FINAL REPORT

Validation of a Novel Bioassay for Low-level Perchlorate Determination

ESTCP Project ER-201030

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Abbreviations and Acronyms

ANOVA   analysis of variance

AU   absorbance units

Cld, cld   chlorite dismutase

DPRB   dissimilatory perchlorate-reducing bacteria

DTAB   decyltrimethyl ammonium bromide

EDQW   Environmnetal Data Quality Workgroup

EPA   Environmental Protection Agency

ESI   electrospray ionization

IC   ion chromatography

ICPMS   inductively coupled plasma mass spectrometry

JPL   Jet Propulsion Laboratory

LC/MS   liquid chromatography/mass spectrometry

LLNL   Lawrence Livermore National Laboratory

LRB   laboratory reagent blank

MDS   multidimensional scaling

MMR   Massachusetts Military Reservation

MOPS   morpholinepropane sulfonic acid

MRL   method reporting level

MS   mass spectrometry

NADH   reduced nicotinamide adenine dinucleotide

NAS   National Academy of Sciences

Pcr, pcr, PCR perchlorate reductase

PD   percent difference

PI   principal investigator

PMS   phenazine methosulfate

PMSH   reduced phenazine methosulfate

PR   plate reader

QA   quality assurance

QC   quality control

RSD   relative standard of deviation

SAM   standard additions method

SPE   solid phase extraction

SDVB   styrene divinylbenzene

UCB   University of California, Berkeley

xt   cell-free extract

Acknowledgements

EXECUTIVE SUMMARY

The bioassay for low-level perchlorate determination (range approximately 6 to 40 µg/L), which was the focus of the project, is a benchtop enzyme assay combined with a perchlorate concentration and purification step using solid phase extraction (SPE) cartridges (Heinnickel et al., 2011). It is based on perchlorate reductase activity and couples NADH oxidation to perchlorate reduction, with PMS as an electron shuttle. Hence the amount of perchlorate in an aqueous sample can be determined enzymatically by monitoring the amount of NADH oxidized spectrophotometrically (i.e. by measuring the decrease in absorbance at 340 nm). The instrumentation for the bioassay is a UV spectrophotometer. The equipment and materials are relatively inexpensive and the bioassay potentially may be performed by less highly trained personnel than required by IC or LC/MS analytical methods. Thus the benchtop bioassay might supplement more expensive and time consuming analytical methods as a screening test for perchlorate in groundwater to facilitate tasks such as mapping plumes and monitoring perchlorate levels during remediation. The goal of the project was to determine whether results with the benchtop bioassay were in agreement with those of a reference analytical method using diverse groundwater sources. If results with the benchtop bioassay performed in the PI's laboratory were comparable to reference analytical methods, it was planned to develop and test a kit format of the bioassay that could be used in the field.

The enzymatic bioassay for perchlorate was developed under SERDP Project ER 1530[19]. Bioassay results with groundwater collected from three different locations at one site were in good agreement with results obtained by a reference IC method. The levels of perchlorate in these samples were approximately 10, 22, and 71 µg/L. This testing was performed in a 96 well microplate using a microplate reader inside of an anaerobic chamber with an atmosphere of nitrogen and hydrogen. In addition, the benchtop version of the bioassay was developed, using quartz cuvettes, to be performed in ambient laboratory conditions. The benchtop bioassay format performed as well as the microplate reader format with perchlorate standards.

Since different groundwater samples vary in the efficiency of perchlorate recovery at the SPE step, subsamples are spiked with perchlorate according to the standard additions method for quantitating an analyte[14]. After applying perchlorate-spiked groundwater to SPE cartridges, the cartridges are rinsed, and then perchlorate is eluted with an alkaline solution (pH >12) of MOPS and NaCl. The eluates are neutralized to about pH 7.2, added to cuvettes, overlaid with mineral oil in order to provide an aqueous compartment separated from air, and NADH and PMS reactants are added. Anaerobic conditions needed for enzyme activity are generated using the non-enzymatic reduction of PMS by NADH in order to reduce dissolved oxygen to water prior to the addition of enzyme. Addition of enzyme initiates the enzyme reaction. An extract of the perchlorate reducing bacterium *Dechloromonas agitata* strain CKB, which contains the enzyme perchlorate reductase, provides the enzyme source for the assay.

The three performance objectives of the project were as follows (see Table 3-1). 1. Compare benchtop bioassay results from testing in the PI's laboratory to results from a reference analytical method performed by a commercial laboratory. 2. Compare results with a benchtop bioassay kit format tested by site field personnel to the site's routine perchlorate detection method. 3. Evaluate the ease of use of the benchtop bioassay kit format.

Regarding objective 1, although it proved difficult to find partners who could collect the groundwater needed for the study, six groundwater sources were obtained for the project. Success criteria were met for one groundwater source, but were not met for four other groundwater sources. See Table 5-4 for a summary of testing outcomes. Quantitative success criteria could not be applied to the sixth source since the amount of ClO_4^- by the reference method was <MRL. Results for this source were considered to have qualitatively met success criteria. Thus the benchtop bioassay, at least in its current format, is not suitable as a screening test for perchlorate in groundwater.   Indepth analysis indicated that oxygen contamination of the bechtop assay, even in the face of several barriers and an active chemical reductant, was the culprit for the unpredictability and poor performance of the bioassay comparison under objective 1. Retesting of the water samples with the bioassay in an anaerobic chamber with a nitrogen/hydrogen atmosphere confirmed this finding and provided a different outcome. In this instance the bioassay accuracy met all performance criteria for groundwater eluates amenable to quantitative testing. Since performance of the bioassay was not high enough to warrant proceeding with development of a kit format for the benchtop bioassay, no testing was done relative to performance objectives 2 and 3. The focus of activities shifted to attempting to identify factors that interfere with the benchtop bioassay in order to determine whether operations and performance could be improved.

It is useful to consider in more detail the performance of the benchtop bioassay with the five groundwater sources that could be quantitatively compared. In addition to the reference method that was performed in a commercial laboratory, analytical testing for perchlorate according to method EPA 314.0 was performed in the PI's laboratory and included in the comparison of methods. The metric used to evaluate accuracy was the percent difference (PD) between results with a method of interest and the results with the reference method tested in a commercial laboratory. A 30 % or less difference was considered to be acceptable accuracy. In addition, for instances where accuracy was acceptable, an ANOVA statistical analysis was used to further evaluate whether bioassay results were significantly different from the reference method.

The benchtop bioassay accuracy was qualatively acceptable for the JPL source. The accuracy was quantitatively acceptable for the Fontana Water Co. source and boarderline for the Hill AFB source. ANOVA testing showed that the methods were not significantly different with the Fontana Water Co. source, whereas they were significantly different for Hill AFB. Additional analysis by the Tukey Method indicated that the benchtop bioassay results were responsible for the difference among the methods for the Hill AFB groundwater. Thus, benchtop bioassay results with Hill AFB groundwater were significantly different from the reference method. Accuracy of the benchtop bioassay was not acceptable (i.e > 30 % PD) for the three other sources: NWIRP McGregor, Massachusetts Military Reservation (MMR), and LLNL. There was a pronounced lack of agreement among replicates tested in the benchtop bioassay for NWIRP McGregor and Massachusetts Military Reservation sources. The reason for this poor performance was not determined but may be due to inefficient reduction of reaction mixtures during the preincubation period before the addition of cell extract. By contrast, with LLNL groundwater, there was good agreement among replicates in the benchtop bioassay testing. Apparently there was interference with the benchtop bioassay seen with the LLNL groundwater. No factor was identified as being responsible for the interference behavior, and interference was

apparently not observed when some LLNL eluate sets were retested using the anaerobic plate reader bioassay format.

It was instructive to retest representative SPE eluate standard additions method sets in the alternative bioassay format, i.e. in a microplate reader in an anaerobic chamber with a nitrogen/hydrogen atmosphere. The accuracy was acceptable for the five groundwater eluates amenable to quantitative testing: Hill AFB, NWIRP McGregor, Fontana Water Co., Massachusetts Military Reservation, and LLNL (Table 5-6). ANOVA and further testing by the Tukey Method (Table 5-7) indicated that the plate reader bioassay and the reference method in a commercial laboratory were not significantly different. Possibly the more reducing conditions used for the plate reader format of the bioassay are responsible for the difference in performance compared to the benchtop format.

In addition to perchlorate, it was shown by ICPMS analysis of groundwater sources and corresponding SPE eluates that other components in groundwater samples are concentrated in SPE eluates. In particular, uranium was concentrated almost to the same degree as perchlorate. Since the level of uranium in SPE eluates of LLNL groundwater was about 9 µM, it seemed possible that uranium might contribute to the interference seen with LLNL groundwater in the benchtop bioassay. However, when uranium (VI) was tested for possible interference with the benchtop bioassay, no effect on perchlorate reductase activity was seen.

1.0   INTRODUCTION

1.1   BACKGROUND

Perchlorate (ClO_4^-) is principally a synthetic compound with a broad assortment of industrial applications ranging from pyrotechnics to lubricating oils[12]. Ammonium perchlorate represents 90% of all perchlorate salts manufactured and is used as an energetic booster or oxidant in solid rocket fuels. Its presence in the environment poses a potential health threat and primarily results from legal historical discharge of unregulated manufacturing waste streams, disposal pond leaching, and the periodic servicing of military inventories. Although a powerful oxidant, under most environmental conditions, perchlorate is highly stable and non-reactive due to the high energy of activation associated with its reduction. Because of the large molecular volume and single anionic charge, perchlorate also has a low affinity for cations and as a result, perchlorate salts are generally highly soluble and completely dissociate in aqueous solution. Perchlorate does not sorb to any significant extent to soils or sediments and, in the absence of any biological interactions, its mobility and fate are largely influenced by the hydrology of the environment[16].

Perchlorate is known to affect mammalian thyroid hormone production and its primary toxicity results from its structural similarity to iodate, which plays an important regulatory role in hormone production by the thyroid gland[3, 18]. Perchlorate readily enters into the food web and can be taken up by forage and edible vegetation[9] as well as plants grown hydroponically[7]. In certain plant species, including tobacco and lettuce, perchlorate accumulates and can persist during processing into final consumer products such as cigarettes and chewing tobacco[8]. Of great concern is a study done in the US on breast and dairy milk showing the presence of perchlorate in almost all samples analyzed[11].

It has been known for more than fifty years that microorganisms can reduce oxyanions of chlorine such as chlorate (ClO_3^-) and perchlorate [collectively denoted (per)chlorate] under reducing conditions. The high reduction potential makes them ideal electron acceptors for microbial metabolism and specialized microbes can grow by the anaerobic reductive dissimilation of (per)chlorate to innocuous chloride. Many dissimilatory (per)chlorate-reducing bacteria (DPRB) have been isolated from a broad diversity of environments including both pristine and contaminated soils and sediments[1, 4, 6]. Remediation strategies for perchlorate contamination include making use of DPRB both in-situ and ex-situ, in addition to other technologies[5].

The successful implementation of any remediative strategy is dependent on the accurate identification of the boundaries of the perchlorate plume. Analytical methods currently available include ion chromatography (IC) with conductivity detection, which forms the basis of EPA Method 314.0, and liquid chromatography or IC coupled with electrospray ionization (ESI) mass spectrometry (MS), which forms the basis of EPA Method 6850 or 6860, respectively. These techniques are laborious, expensive, time consuming, and require highly trained personnel, making them unsuitable for the rapid delineation of contaminated environments. An alternative biochemical technique was developed under SERDP Project ER 1530 "An Enzymatic Bioassay for Perchlorate" by Coates, Heinnickel, and Achenbach, 2010[19], which is based on perchlorate reductase activity coupled to NADH oxidation, with PMS as an electron shuttle. The

instrumentation for the bioassay is UV spectrophotometer used to measure the absorbance of NADH at 340 nm.

Bioassay results with groundwater samples from three wells at one collection site were in good agreement with results obtained by a reference IC method. The levels of perchlorate in these samples were approximately 10, 22, and 71 ppb. This testing was performed in a 96 well microplate using a microplate reader inside of an anaerobic chamber with an atmosphere of nitrogen and hydrogen. In addition, a benchtop version of the bioassay was developed using quartz cuvettes to be performed in ambient laboratory conditions. The benchtop bioassay format performed as well as the microplate reader format with perchlorate standards. Thus the benchtop bioassay method appeared to have the potential to supplement more expensive and time consuming analytical methods as a screening test to facilitate tasks such as mapping plumes and monitoring perchlorate levels during remediation.

1.2   OBJECTIVE OF THE DEMONSTRATION

The overall project goals of ER-201030 were: 1. Evaluate the sensitivity and reliability of the benchtop perchlorate bioassay as a screening test for perchlorate in the 6 to 40 ppb range, compared to IC or LC/MS analytical techniques as the reference method, using groundwater samples from diverse environments. If bioassay results were consistent with those of the reference method, thereby showing promise as an alternative screening test for perchlorate, proceed to the second goal. 2. Assess the feasibility of using the bioassay in a kit format to monitor perchlorate levels at field sites by developing reagents, an SOP, and providing technical support to field personnel unfamiliar with the assay who would test samples by the bioassay in parallel with the site's usual analytical procedures. Bioassay results would be compared to results from the site's routine perchlorate testing method, and the ease of use would be evaluated by questionnaire and discussions with field testing personnel.

Six sites provided groundwater to meet the first goal. The benchtop bioassay met the criterion for accuracy relative to the reference analytical method for only one groundwater source, and accuracy was qualitatively acceptable with a second source. With a third source, bioassay results did not meet the accuracy criterion, although they were close to the cut-off for acceptance. In addition, bioassay results for this source were significantly different from the reference analytical method by an ANOVA analysis. Finally, the benchtop bioassay accuracy was unacceptable for an additional three groundwater sources. Thus the benchtop bioassay, at least in its current format, is not suitable as a screening test for perchlorate in groundwater. Since performance of the bioassay was not high enough to warrant proceeding with field testing of the bioassay in a kit format, the focus of activities shifted to attempting to identify factors that interfere with the benchtop bioassay in order to determine whether operations and performance could be improved.

1.3   REGULATORY DRIVERS

Before 1997, perchlorate was unregulated in the US, however, its discovery in national drinking water resources, prompted the establishment of a federal provisional action level of 18 parts per billion (ppb or µg/L) resulting in a decade of high profile debate over the determination of a final federal action level. In 1998 perchlorate was added to the US EPA Contaminant Candidate List

for drinking water supplies[17], and a final decision regarding the regulatory limit was to be set pending the outcome of ongoing toxicological studies. States such as Massachusetts, New Jersey, and California, adopted their own regulatory levels of 2, 5, and 6 ppb, respectively, in an attempt to limit the health impact of this contaminant while awaiting the outcome of a federal decision[13]. In January 2002, as a result of publication of the first draft of the US EPA review on toxicological and risk assessment data, a revised and lowered health protective standard of 1 µg/L was suggested. Since the findings of this draft assessment were highly controversial to three other federal agencies, the US National Academy of Sciences (NAS) was asked to make an assessment. In January of 2005 the NAS suggested a maximum permissible dose of 0.7 µg/kg/d. This suggestion correlates to a standard of about 23 µg/L for a normal adult person. However, the level would be lower for infants and children based on weight. This is especially poignant due to the recent study done on breast and dairy milk in the US[11]. The highest level detected in breast milk was 92 µg/L, a level 20 times higher than the NAS estimated maximum permissible dose for a baby. Reports of this magnitude are pressuring officials to set a final regulatory limit in the near future, and focus interest on technologies that support perchlorate remediation at contaminated sites. The current EPA Interim Drinking Water Health Advisory for perchlorate exposure is 15 µg/L[24].

2.0   TECHNOLOGY

The perchlorate bioassay uses an extract of the perchlorate reducing bacterium *Dechloromonas agitata* strain CKB, which contains the enzyme perchlorate reductase. The bioassay is based on coupling perchlorate reduction to NADH oxidation using phenazine methosulfate (PMS) as an electron shuttle to transfer electrons from NADH to the perchlorate reductase enzyme[2]. Hence the amount of perchlorate in an aqueous sample can be determined enzymatically by the decrease in absorbance at 340 nm. The method also requires prior perchlorate concentration and purification using solid phase extraction (SPE) cartridges by a modification of the procedure described by Thorne[15]. Since different samples vary in the efficiency of perchlorate recovery at the SPE step, subsamples are spiked with perchlorate according to the standard additions method for quantitating an analyte[14]. Cartridges are rinsed and then perchlorate is eluted with an alkaline solution (pH >12) of morpholinepropane sulfonic acid (MOPS) and NaCl. The eluates are neutralized to about pH 7.2, added to cuvettes, and anaerobic conditions needed for enzyme activity are generated using the non-enzymatic reduction of PMS by NADH in order to reduce dissolved oxygen to water.

2.1   TECHNOLOGY DESCRIPTION

The benchtop bioassay method for low-level perchlorate determination is a UV spectrophotometric enzyme assay with a prior perchlorate concentration and purification step using solid phase extraction (SPE) cartridges[2]. The equipment and materials are relatively inexpensive and the bioassay potentially may be performed by less highly trained personnel than required by IC or LC/MS analytical methods.

The SPE step uses cartridges and a cartridge conditioning procedure previously described by Thorne (2004) both to concentrate perchlorate[15] and lower the levels of other anions that would interfere with subsequent perchlorate detection, such as nitrate and chlorate (ClO_3^-). In addition, a standard additions method[14] is used to compensate for the variable recovery of perchlorate from SPE cartridges. Hence, a 1 L sample of groundwater for bioassay analysis is divided into five 200 mL subsamples. One subsample is not spiked with any perchlorate and the remaining four are spiked with increasing increments of 10 ppb (parts per billion, i.e. µg/L) of perchlorate. Each subsample is applied to a separate conditioned SPE cartridge. To condition cartridges prior to use they are sequentially rinsed with acetone, deionized water, and decyltrimethyl ammonium bromide (DTAB). After a 200 mL subsample is passed through a cartridge, the cartridge is rinsed with 7.5 mL of a solution of 2.5 mM DTAB in 15% acetone in water in order to remove chlorate (ClO_3^-), nitrate and other anions. Then perchlorate is eluted using 2 mL of a solution (pH > 12.0) containing 200 mM (MOPS) and 2 M NaCl. Perchlorate potentially could be concentrated 100 fold in eluates compared to the starting levels in subsamples. Figure 2-1 shows a schematic diagram illustrating how groundwater samples were tested by the bioassay method in a benchtop format as well by reference analytical methods.
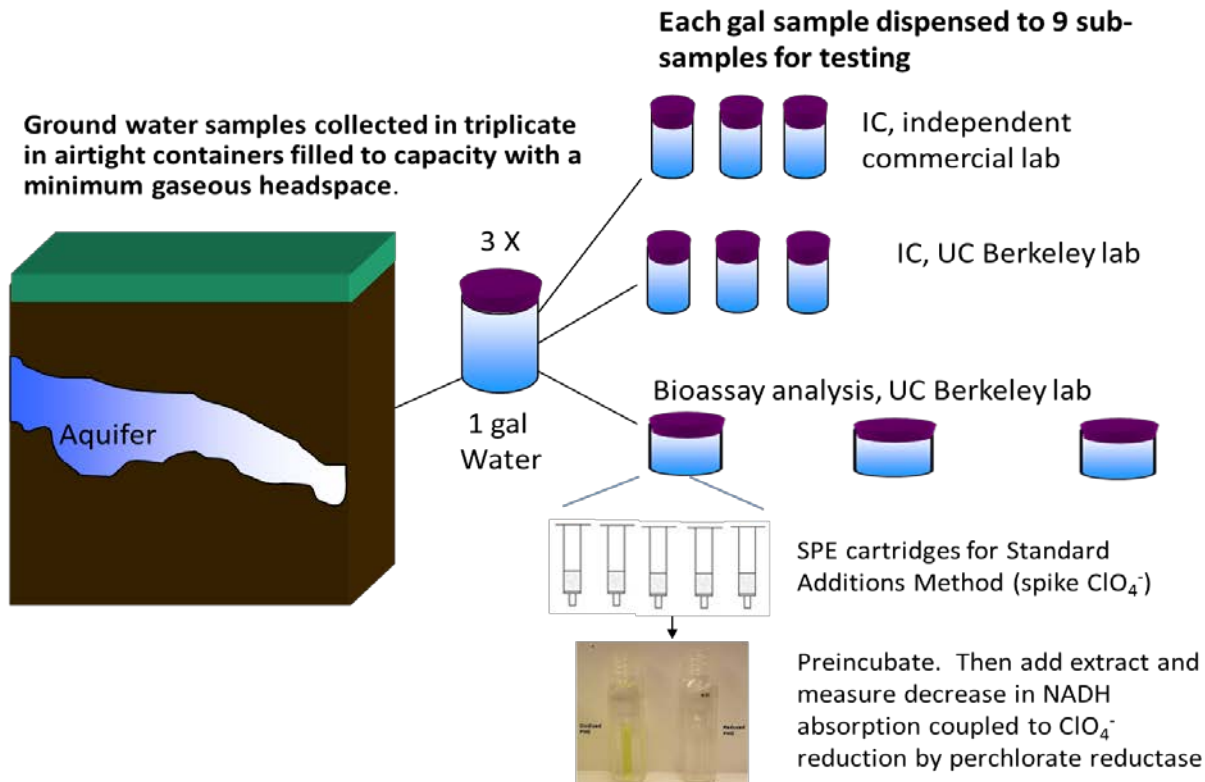
Figure 2-1. Schematic diagram of groundwater testing by benchtop bioassay and reference analytical methods

In preparation for the bioassay enzyme step, HCl is added to MOPS-NaCl eluates to adjust the pH to about 7.2, which is the pK_a for a MOPS buffer and ideal for perchlorate reductase activity. 1 mL of this perchlorate sample solution is placed in a quartz cuvette and overlaid with mineral oil to exclude air. Other components of the reaction mixture are then added to provide for both non-enzymatic removal of oxygen during a preincubation interval (see Figure 2-2) and reactants for perchlorate reductase activity (Figure 2-3) when extract is subsequently added to reaction

Figure 2-2. Non-enzymatic reduction of O_2 by NADH and PMS

mixtures. NADH quickly reduces PMS non-enzymatically, which yields oxidized NAD^+ and reduced PMS. This initial non-enzymatic reduction of PMS is easily observed operationally, since oxidized PMS is yellow, whereas reduced PMS is colorless. In addition to the color change, reaction mixtures also become turbid because the reduced PMS is less soluble than oxidized PMS. The cuvette on the left in Figure 2-1 has PMS in the oxidized state, whereas the cuvette on the right has reduced PMS. Subsequent non-enzymatic reduction of oxygen to water, accompanied by reoxidation of PMS, apparently is a much slower reaction. This proved to be a significant impediment for the benchtop bioassay method, since perchlorate reductase requires

and anaerobic environment and reaction mixtures must be rendered anoxic before enzyme is added.

Figure 2-3. Perchlorate reductase coupled to NADH oxidation
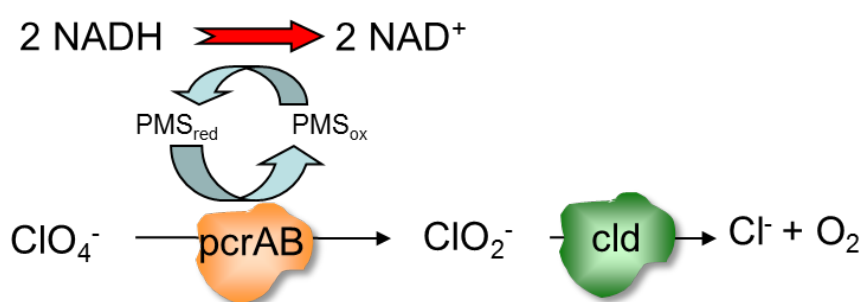
The enzyme reaction is initiated by adding extract from the CKB strain of *Dechloromonas agitata*, which contains the perchlorate reductase (Pcr) enzyme. PMS and the remaining NADH are then used in the enzymatic reaction to reduce perchlorate.

Figure 2-3 shows the components of the NADH-coupled enzyme assay for perchlorate. The reduced PMSH shuttles electrons to PCR so that the perchlorate is reduced to chlorite (ClO_2^-) by the enzyme. NADH doesn't interact with the PCR enzyme directly, but is coupled to the PCR reaction through the PMS shuttle component. Because extracts also contain the enzyme chlorite dismutase (Cld), the chlorite PCR product can be dismuted to Cl^- and O_2. The O_2 generated in the chlorite dismutase reaction can in turn be non-enzymatically reduced by the NADH and PMS (Figure 2-2). Thus the overall stoichiometry when both enzymes are active would be 4 moles NADH oxidized per mole of perchlorate reduced to Cl^-.
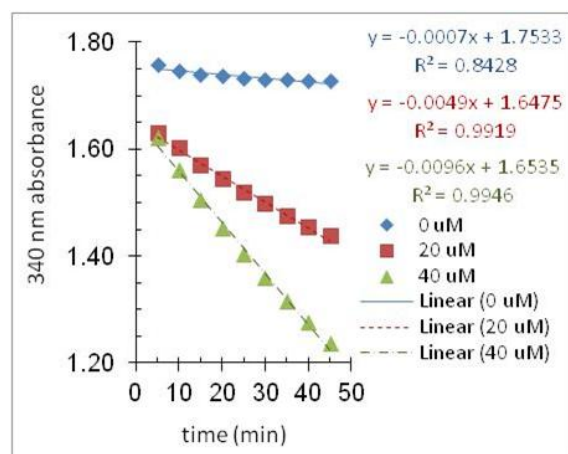
Figure 2-4. Perchlorate reductase activity in reactions with different starting concentrations of perchlorate

The oxidation of NADH is measured spectrophotometrically at 340 nm since NADH, but not NAD^+, absorbs at this wavelength. As illustrated in Figure 2-3 and discussed above, for each mole of perchlorate reduced, two to four moles of NADH are oxidized. Reactions are incubated at room temperature for about 55 minutes and absorbance measurements are made at 340 nm. The time at which extract is mixed with the other components in a cuvette is taken as the initial time. Absorbance is then measured every 5 minutes from about 10 to 55 minutes to determine the rate of reaction (i.e. the change in 340 nm absorbance/minute). It was envisioned that the kit for field bioassay testing would make use of only two absorption determinations. The first would be after about 10 minutes of reaction and the second after 45 or 55 minutes. For CKB cell extracts with perchlorate reductase activity, the rate of absorption decrease at 340 nm is correlated to the concentration of perchlorate present initially in the reaction mixture (see Figure 2-4). Samples can be analyzed batchwise so that results are obtained for a number of samples simultaneously.

Figure 2-5. Determination of perchlorate in a groundwater sample by the standard additions method

Data from the enzyme assay of spiked subsamples are used to determine the amount of perchlorate in a groundwater sample. The rate of reaction (i.e. the change in 340 nm absorbance/minute) is plotted on the y-axis and the amount of perchlorate spike (μg/L) on the x-axis. By the standard additions method, the concentration of perchlorate in the sample equals (-1) times (x-intercept)[14]. Figure 2-5 shows a plot for the determination of perchlorate in SPE eluates of a groundwater sample. In this example, the amount of perchlorate was 9.9 ± 2.0 μg/L.

A summary of results comparing the perchlorate benchtop bioassay with reference methods is listed in Table 5-4.

2.2   TECHNOLOGY DEVELOPMENT

The bioassay for perchlorate used for this project was developed during SERDP Project ER 1530[19], also described in Heinnickel et al., 2011[2]. The bioassay makes use of perchlorate reductase, an anaerobic enzyme. Briefly, an earlier assay for perchlorate reductase depended on the use of methyl viologen as the reductant and electron shuttle, and is described in Kengen et al., 1999[10]. However, investigation of this method in ER 1530 indicated that it was unreliable for low perchlorate levels. It was shown in ER 1530 that instead of methyl viologen, NADH could be used as the reductant and that this change was advantageous in several key respects:

1. The empirical ratio of NADH oxidized to perchlorate reduced is similar to theoretical perdiction so that 340 nm absorbance change (i.e. the amount of NADH oxidized) and the amount of perchlorate reduced are quantitatively coupled.
2. NADH is stable to air so that all the necessary reactants for the enzyme reaction (i.e. perchlorate, NADH, and PMS) can be stored in air under ambient conditions.
3. Reaction mixtures must be made anaerobic prior to adding the perchlorate reductase enzyme, which is sensitive to air and requires anaerobic conditions for activity. NADH and PMS react non-enzymatically and could be used to remove oxygen from enzyme reaction mixtures prior to the addition of enzyme.

In addition, several other crucial features were incorporated into the developed bioassay. A cleared cell extract supernatant, rather than purified enzyme protein, is suitable as the enzyme source. This reduces the time and cost of providing enzyme reagent. A pretreatment step using DTAB conditioned SDVB in SPE cartridges, can effectively concentrate perchlorate from groundwater samples. This use of SPE cartridges, previously developed by Thorne[15], was modified by using an alkaline MOPS solution instead of acetone to elute perchlorate from the cartridges. The eluates were suitable for use in perchlorate reductase reaction mixtures after neutralization to pH 7.0 to 7.2 and removal of oxygen. Nitrate and chlorate anions, which are alternative substrates for perchlorate reductase, are removed at the SPE step. Because the

efficiency of perchlorate recovery from SPE cartridges was known to be variable[21] due to groundwater matrix variations, a standard additions method was adopted for the bioassay procedure whereby five 200 mL portions of groundwater were spiked with different amounts of perchlorate (i.e. 0, 10, 20, 30, or 40 μg/L) prior to application to SPE cartridges. Inclusion of 2 M NaCl in the alkaline MOPS eluting solution also was useful in several respects: lowering the background of other NADH oxidizing enzyme reactions, decreasing the solubility of oxygen in eluates, and decreasing diffusion of oxygen into the reaction mixture from the walls of the cuvette. Environmental samples were collected from different locations at the Aerojet Sacramento, CA, site and donated for bioassay analysis. Results with the bioassay (anaerobic plate reader) method were not significantly different from results obtained by a reference IC method[2]. Lastly, a benchtop version of the bioassay was developed in which 1 mL of an enzyme buffer (i.e. MOPS-NaCl solution at about pH 7.2) containing perchlorate could be placed in a quartz cuvette, overlaid with mineral oil to separate the reaction from air, and amended with NADH and PMS to non-enzymatically consume sufficient oxygen during a preincubation period to obtain conditions suitable for perchlorate reductase activity. Reactions were subsequently initiated with the addition of cell extract, and the change in 340 nm absorbance showed a good correlation between the amount of NADH oxidized and the amount of perchlorate reduced[2].

The focus of the current project was on the benchtop bioassay format, first to test environmentally diverse groundwater sources with the bioassay and second, if the performance of the benchtop bioassay was satisfactory, to develop and field test a kit version to be used as a possible perchlorate screening test.

Additional development work was done to improve operations. The benchtop bioassay requires that reactants be added to the aqueous phase under the mineral oil overlay and mixed to obtain a homogeneous solution. Different approaches to mixing were tested. Both repipetting with disposable plastic transfer pipettes and stirring, with disposable inoculating loops or a reusable stirring tool, were satisfactory when tested in reactions with perchlorate standards in enzyme buffer. However, when SPE eluates containing perchlorate were used, stirring seemed to correlate better with less variable non-enzymatic NADH oxidation.

Obtaining sufficiently reduced conditions in reaction mixtures by the non-enzymatic reaction between NADH and PMS proved difficult, particularly when SPE eluates were used. The non-enzymatic oxidation of NADH in the presence of PMS was much greater for the benchtop format than observed when reactions were performed in the anaerobic chamber using the plate reader format. NADH oxidation seen during the preincubation period was variable among replicate SPE eluate sets (see Appendix L, Table L-1) and often of much greater magnitude than occurs during perchlorate reduction by perchlorate reductase. Some consideration was given to the approach of adding reductants, in addition to the NADH and PMS system, to more efficiently reduce SPE eluates prior to bioassay analysis. However, using reductants such as ascorbic acid, dithiothreitol, or dithionite was not attractive, since these reduce PMS[2], and it was thought they would interfere with the stoichiometry of NADH and perchlorate in the bioassay. With regard to the reductant cysteine (an amino acid with a sulfhydral group), PMS is known to chemically react with sulfhydral groups. Mixing oxidized PMS with cysteine resulted in the rapid formation of a red degradation product, showing that cysteine wouldn't be suitable as an additional reductant for the benchtop bioassay. Thus the only approach adopted for benchtop bioassay testing was to extend

the preincubation period during which residual oxygen is removed non-enzymatically and amend reaction mixtures with additional NADH prior to addition of extract to start the enzymatic reaction. Apparently, this procedure was not sufficiently effective or reproducible as a means of making reaction mixtures suitably reduced prior to perchlorate reductase addition.

Development work was also done to improve SPE operations. In the current version of the bioassay, groundwater is applied to SPE cartridges using positive pressure. A condensed nitrogen gas source provides the necessary pressure. Testing was done to determine whether gravity flow would be an effective way of applying samples for perchlorate extraction if large resin beads of SDVB, conditioned with DTAB, were used. 3 g of SDVB (20 % cross-linked) white beads of 20 – 60 mesh were packed into the barrels of 10 mL syringes, conditioned with DTAB, and 200 mL of deionized water containing perchlorate (100 µg/L) was applied by gravity flow. IC analysis of perchlorate in the effluents showed that 96.1 ± 1.6 % of the perchlorate (average of three replicates ± standard deviation) was extracted. (See Appendix K for more detail.) Application of groundwater by gravity flow would be much more convenient and save operator contact time in conducting bioassay testing.

As illustrated in Figure 2-4 of Section 2.1, and studied previously (Coates and Achenbach, unpublished), the rate of 340 nm absorption is correlated to the concentration of perchlorate present initially in the reaction mixture when a CKB extract is used for the bioassay. To evaluate whether this is a general feature of perchlorate reductase enzymes from dissimilatory perchlorate reducing bacteria (DPRB) in the genus *Dechloromonas*, extracts of *D. aromatica* strain RCB were also tested in the bioassay. With the RCB extract, bioassay rates were distinctly less variable at different perchlorate concentrations than is seen with a CKB extract (see Appendix L Section L-5 for more detail). This effect on the rate of reaction was observed in both plate reader and benchtop bioassay formats. Had the development of a kit for the benchtop bioassay gone forward, it might have been advantageous to test RCB extracts for their suitability for that application, since the rate of absorbance change is faster at low perchlorate concentrations when an RCB extract is used.

The stoichiometry of the NADH-coupled perchlorate reductase bioassay was previously studied[19]. In the present study, the stoichiometry was about 4 moles of NADH oxidized per mole of perchlorate reduced using a CKB extract in both the benchtop and plate reader formats (see Appendix L Section L-4 for more detail).

2.3   ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The major advantages of the perchlorate bioassay technology are that the materials and equipment are relatively inexpensive, operators potentially do not need to be highly trained, and results from the assay can be obtained with a short turnaround time.

A limitation to the bioassay methodology is its complexity. There are three component steps to the overall procedure: use of SPE cartridges to concentrate and purify perchlorate in spiked subsamples, spectrophotometric determination of perchlorate, and data analysis to calculate the perchlorate concentration in the sample by the standard additions method using linear regression. A great difficulty in the current version of the benchtop bioassay is not having an efficient

method of obtaining anaerobic conditions in reaction mixtures prior to the addition of perchlorate reductase. Perchlorate reductase is not active aerobically. Furthermore, lack of anaerobicity favors non-enzymatic NADH oxidation in the presence of PMS and other components in reaction mixtures, thus compromising the specificity of the benchtop bioassay.

3.0   PERFORMANCE OBJECTIVES

Performance objectives with the corresponding data requirements, success criteria, and results are listed in Table 3-1.

Table 3-1. Performance Objectives, Data Requirements, Success Criteria, and Results

Performance Objective	Data Requirements	Success Criteria	Results
Quantitative Performance Objectives			
1. Compare benchtop bioassay results from testing in the PI's laboratory to results from reference analytical method	Results from testing samples for perchlorate in triplicate using: • Benchtop bioassay performed in the PI's laboratory • EPA Method 314.0 or EPA Method 6850 performed in a commercial laboratory • IC EPA Method 314.0 performed in PI's laboratory (data for troubleshooting)	• The percent difference between bioassay and reference method results (from commercial laboratory testing) is 30% or less. • ANOVA statistical analysis shows that results from benchtop bioassay and reference method are not significantly different.	• Success criteria were met for one groundwater source. • Success criteria were not met for four groundwater sources. See Table 5-4 for a summary of testing outcomes. • Quantitative success criteria could not be applied to a sixth source since the amount of ClO_4^- by the reference method was <MRL. Results for this source were considered to have qualitatively met success criteria.
2. Compare results with benchtop bioassay kit format tested by site field personnel to the site's routine method	• Results from benchtop bioassay testing by site field personnel • Results from testing by the site's routine method	• Controls show bioassay is performed correctly • Student t-test shows results from the benchtop bioassay and the site's routine method are not significantly different.	Testing was not done to evaluate this objective. Testing for performance objective 1 indicated that the benchtop bioassay in its current format is not suitable for use in the field, thus no testing was performed by field personnel.
Qualitative Performance Objective			
Ease of use	Feedback from field personnel on the usability of bioassay kits	Feedback from several field technicians able to perform the bioassay	No feedback was obtained since the benchtop bioassay was not tested by field technicians.

3.1   QUANTITATIVE PERFORMANCE OBJECTIVE 1: COMPARE BENCHTOP BIOASSAY RESULTS TO REFERENCE ANALYTICAL METHOD RESULTS

Quantitative performance objective 1 was to compare results from benchtop bioassay testing performed in the PI's laboratory with results performed by a reference chemical analytical method in a commercial laboratory. The reference analytical methods were either LC/MS tested according to EPA Method 6850, or IC tested according to EPA Method 314.0, which was used for the first three groundwater sources. In addition, IC data were collected in the PI's laboratory according to EPA Method 314.0, which was used to troubleshoot different steps of the bioassay testing procedure.

3.1.1   Data Requirements

Three sets of data – one for each of the three methods being compared – were obtained with each groundwater source in order to satisfy the objective. Specifically, the intent was to have project partners at each site collect three 1 gallon containers of groundwater at a designated sampling location and ship them to the UC Berkeley laboratory. Groundwater from each of the gallon containers was subdivided for testing in triplicate by the three methods of interest: 1. the perchlorate reductase benchtop bioassay method, 2. analysis according to EPA Method 6850 or Method 314.0 in a commercial testing laboratory, and 3. IC analysis according to EPA Method 314.0 in the PI's laboratory. Table 5-4 summarizes the results from the three methods, and Table 5-5 has the ANOVA testing. With respect to the testing performed by commercial laboratories, the first three groundwater sources were analyzed by IC according to EPA Method 314.0. Subsequent groundwater sources were analyzed by LC/MS according to EPA Method 6850 because of its greater specificity and sensitivity[20].

The target number of groundwater sampling locations to be used in this component of the study was 10, however, it was only possible to obtain groundwater for the project from 6 locations.

3.1.2   Success Criteria

The accuracy of benchtop bioassay estimates of the perchlorate concentration in different groundwater sources was evaluated by comparison to results from the reference analytical method performed in a commercial laboratory. The metric used to determine whether the bioassay level of accuracy was acceptable was the percent difference (i.e. the absolute value of the difference between bioassay and reference method values ÷ the reference method value, expressed as a percent). The criterion for acceptable accuracy was a percent difference (PD) of 30 % or less. In addition, when the accuracy of the bioassay met the acceptance criterion, ANOVA analysis was then used to provide a statistical assessment of possible differences among results obtained by the three methods. The percent confidence interval at which results were deemed significantly different was 95%, i.e. the level of $\alpha = 0.05$ was used.

Furthermore, a statistical comparison was only possible for perchlorate concentrations at or above the MRL for the reference analytical method. The Jet Propulsion Laboratory (JPL) groundwater sample was intentionally collected from a location having historically low perchlorate, in order to determine whether a false positive would be seen with the bioassay. When the JPL sample was tested with the bioassay, the level of perchlorate was 3 ± 2 µg/L. Perchlorate was not detected by the reference analytical method in the commercial laboratory or by IC at UCB and was thus considered <MRL, i.e. <2 µg/L or <4 µg/L, respectively. In this case, the bioassay result was considered consistent or qualitatively similar to the result obtained by the more sensitive analytical methods even though no percent difference or statistical comparison could be made.

3.1.3   Results

Table 5-4 lists the perchlorate results estimated by the reference method in a commercial laboratory, IC at UCB, and the benchtop bioassay method, as well as the percent difference (PD) values. By the PD comparison, the accuracy of the benchtop bioassay was acceptable for Fontana Water Co. and boarderline for Hill AFB. Results with JPL couldn't be compared quantitatively since the perchlorate from this source was <MRL for the reference method. Since the perchlorate estimated by the benchtop bioassay was low, i.e. 3 µg/L, results were considered qualitatively acceptable. The benchtop bioassay test failed for McGregor NWIRP, Massachusetts Military Reservation (MMR), and Lawrence Livermore National Laboratory (LLNL) groundwater sources, i.e. the PD values were 325, 429, and 92 %, respectively. In most cases, the variation among replicates was greater for the benchtop bioassay than for the other analytical methods. However, the variation was pronounced for the McGregor and MMR testing where relative standard deviations (i.e. the standard deviation ÷ average, expressed as a percent) were 118 and 96 %, respectively. In addition, the average perchlorate estimated with the bioassay was 4 to 5 fold greater than for the reference method, However, for LLNL testing, the average perchlorate estimated with the bioassay was 13 fold lower than for the reference method, and there was good agreement among replicate SPE eluate sets, suggesting there may have been inhibition of the bioassay with this source.

ANOVA was performed using using the Hill AFB and Fontana Water Co. data (see Table 5-5). This analysis showed that the three methods were not significantly different for Fontana Water Co. groundwater. However, the benchtop bioassay was significantly different from the analytical methods for the Hill AFB source.

3.2 QUANTITATIVE PERFORMANCE OBJECTIVE 2: COMPARE BENCHTOP BIOASSAY FIELD KIT RESULTS TO SITE'S REFERENCE METHOD RESULTS

Results with the benchtop bioassay did not justify the development of a field kit, hence no testing was done relative to this objective.

3.3 QUALITATIVE PERFORMANCE OBJECTIVE: FIELD KIT EASE OF USE ASSESSMENT

No field kit was developed, hence no assessment was done relative to this objective.

4.0 SITE DESCRIPTION

4.1 SITE SELECTION

The criteria that were used in site selection are listed in Table 4-1. Despite an extended search on the part of both the liaison officer and the PI, it was only possible to identify six sites as groundwater sources for the project.

Table 4-1. Site Selection Criteria

Parameter	Preferred Value(s)	Relative Importance (1-5, with 1 being the highest)
Known range of perchlorate concentration	0 to 100 µg/L	1
Availability of historical hydrological data regarding variability of the zone of sample collection	Yes	2
Availability of historical data on geochemistry (major anions, cations, and organic constituents), field parameters (DO, ORP, pH, conductance), and the presence of co-contaminants (RDX, etc.)	Yes	2
Availability of local shipping facilities	Yes	1
Reasonable site access and personnel available for sample collection	Yes	1

4.2 SITE LOCATION AND HISTORY

The sites of groundwater sources are listed in Table 4-2, which briefly summarizes some current or past activities characteristic of missions served by these sites and the range of historical perchlorate levels that have been reported at various locations on the site. Sampling locations chosen for collecting groundwater to use in bioassay testing were sufficiently distant from any ongoing remediation activities that no impact on perchlorate concentration was likely.

Table 4-2. Sites of Groundwater Sources

Project No.	Site	Site Location	Current Site Mission or History	Comments on Historical Perchlorate Testing
102	Hill Air Force Base	Ogden, UT	F-16 fighter planes and Minuteman ICBs logistics management	Groundwater 3.6–36 µg/L depending on sampling location
103	Former Naval Weapons Industrial Reserve Plant McGregor	McGregor, TX	Produced solid propellant rocket motors	Navy returned plant to McGregor in 2006. In situ biological treatment system. Groundwater, 2007: 1,300 µg/L in 4 of 39 samples.
104	Jet Propulsion Laboratory (JPL)	Pasadena, CA	Research and development center	Location selected as the project groundwater source historically had no perchlorate
105	Fontana Water Company	Fontana, CA	Municipal drinking water supply	Location selected as the project groundwater source is a production well
106	Massachusetts Military Reservation (MMR)	Cape Cod, MA	Military training facility (Coast Guard, and Army and Air Force National Guard)	Groundwater as high as 200 µg/L, RDX is a co-contaminant at some sampling locations.

Project No.	Site	Site Location	Current Site Mission or History	Comments on Historical Perchlorate Testing
107	Lawrence Livermore National Laboratory (LLNL)	Site 300, Tracy, CA	Explosives test site	Range of concentrations in groundwater, including several wells in the 4 to 40 µg/L range.

4.3   SITE GEOLOGY/HYDROGEOLOGY

Historical information on the geology, hydrogeology, and matrix of sampling locations was considered in order to have groundwater samples as diverse as possible included in the testing.

4.4   CONTAMINANT DISTRIBUTION

Historical data on the variability in perchlorate concentration were reviewed to assist in selecting sampling locations that had as low a variability in perchlorate concentration as possible. When possible, locations likely to provide groundwater samples that contained co-contaminants in addition to perchlorate were included in the testing. The ideal perchlorate concentration range was 0 – 40 µg/L. Three locations (JPL, Fontana Water Co., and MMR) had historical perchlorate concentrations less than 10 µg/L.

5.0   TEST DESIGN

No new sampling installations were used for this study. Instead, the aim was to coordinate the collection of samples to coincide with the routine groundwater sampling schedule of the different locations included in the study. Groundwater from the different sites was tested to evaluate the performance of the bioassay on various kinds of samples.

5.1   CONCEPTUAL EXPERIMENTAL DESIGN

Objective one was to perform bioassay testing on environmentally diverse groundwater samples, defined as groundwaters from a diverse assortment of geographic regions expected to differ with respect to geochemical parameters such as pH, salinity, iron content, temperature, and conductivity. However, the groundwater sources included in the study were collected primarily based on availability. Three labeled gallon containers were collected at each sampling location (see Figure 2-1). These were cooled and shipped overnight along with a chain-of-custody form to the UC Berkeley laboratory where the integrity of the samples was examined and temperature, pH, and conductivity were measured. Each gallon of groundwater was filtered to remove particulate material and subdivided to obtain nine subsamples. The subsamples to be tested by a commercial laboratory were shipped along with chain-of-custody documentation to the testing facility. Subsamples were subsequently tested for perchlorate in triplicate by each method (i.e. benchtop bioassay, a reference analytical method performed by a commercial laboratory, and IC according to EPA method 314.0 at the UC Berkeley laboratory).

Residual sample from each gallon container was stored at 2-8 °C in case additional analytical testing was desirable to identify factors in a sample that might influence bioassay performance.

5.2   BASELINE CHARACTERIZATION

Historical data on the perchlorate concentration at sampling locations over several years was used to help select the locations for groundwater sampling. No additional baseline characterization activities were undertaken.

5.3   TREATABILITY OR LABORATORY STUDY RESULTS

No treatability or laboratory scale studies were part of the project.

5.4   FIELD TESTING

Testing of the perchlorate benchtop bioassay was not carried out since the assay in its current format is not suitable as a screening test for use in the field.

5.5   SAMPLING AND ANALYTICAL METHODS

The number of groundwater samples collected for the project and the types of tests performed are summarized in Table 5-1. The analytical methods are listed in Table 5-2. Appendix E

includes information describing the calibration of analytical equipment, quality assurance sampling, decontamination procedures, and sample documentation.

Table 5-1. Total Number of Groundwater Samples Collected for Benchtop Bioassay, Perchlorate Reference Methods, and Characterization Testing

Component	Matrix	No. of Samples	Analyte	QC/QA	Comment or Location
Bioassay performed in PI's laboratory, and reference analytical methods (EPA 6850 or 314.0 in commercial laboratory, and EPA 314.0 in PI's laboratory)	Ground-water	17[a]	Perchlorate	Bioassay: include positive and negative controls as well as track enzyme activity in each analysis batch. Analytical methods: confirm instrument performance parameters and include duplicates, spikes and other controls per EPA methods.	6 sites provided groundwater. Each gallon sample was tested in triplicate for bioassay as well as EPA 314.0 in the PI's laboratory and by reference methods (EPA 314.0 or 6850) in commercial laboratories.
	Ground-water	17[a]	pH and conductive-ity	Confirm instrument performance parameters per instrument manuals.	All samples were tested in PI's lab or at the time of collection.
	Ground-water	As needed	Physical and chemical parameters, listed in Table 5-2[b]	Confirm instrument performance parameters per instrument manuals. For IC methods include laboratory duplicates and matrix spikes.	When there was a lack of agreement between bioassay and reference method, more factors were evaluated for possible effects on bioassay performance.

[a]Each sampling event consisted of collecting three replicate gallons of groundwater. Thus the total number of sample bottles to be tested was 5 x 3 = 15 plus 2 bottles from the NWIRP McGregor location.
[b]Physical and chemical parameters measured included ionic species analyzed by IC (such as nitrate, chlorate, chloride, phosphate, fluoride, nitrite, sulfate, and bromide), and analysis of elements by ICPMS.

5.5.1 Analysis Methods

Analysis of perchlorate by the reference analytical methods by commercial laboratories was performed by TestAmerica Laboratories, Inc. and Calscience Environmental Laboratories, Inc. Elements were analyzed by inductively coupled plasma mass spectrometry using a Perkin Elmer SCIEX Elan DRC II Inductively Coupled Plasma Mass Spectrometer (ICPMS). Analyses were conducted at the Lawrence Berkeley National Laboratory by the Analytical Service Center of the Aqueous Geochemistry Laboratory in the Earth Sciences Division.

Detailed operations for performing the bioassay are described in Appendix L.

Table 5-2. Analytical Methods for Sample Analysis

Matrix	Analyte	Method	Container[a]	Preservative[b]	Holding Time
Ground-water	Perchlorate	Bioassay, EPA 6850, EPA 314.0	LDPE bottle	2-8 °C	28 days
	pH	Electrochemical Probe	LDPE bottle	2-8 °C	Immediately on receipt or at time of collection
	Conductivity	Electrochemical Probe	LDPE bottle	2-8 °C	Immediately on receipt or at time of collection
	Common anions[c]	IC	LDPE bottle	2-8 °C	Not specified
	Common elements	ICPMS	LDPE bottle	2-8 °C	Not specified
	Uranium	ICPMS	LDPE bottle	2-8 °C	Not specified

[a]One gallon container holds sufficient groundwater to test for perchlorate by bioassay, IC, and LC/MS as well as to determine pH, conductivity, and physical/chemical parameters.
[b]Preservatives are not required for these analyses, however, all samples were stored at 2 – 8°C and shipped chilled on ice or with cold packs.
[c]Ionic species analyzed by IC included nitrate, chlorate, chloride, phosphate, fluoride, nitrite, sulfate, and bromide

5.5.2 Statistical Analyses

The bioassay determination of perchlorate is by a standard additions method, and (-1) times the x-intercept of the standard additions method regression line provides the concentration of perchlorate in the sample[14]. Linear regression statistical analysis[22] was performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington). Analysis of variance (ANOVA) statistical testing was performed using Minitab 16.2.3 software (Minitab, Inc., State College, Pennsylvania). Multi-dimensional Scaling (MDS) statistical analysis was performed using Primer 6 software (Primer-E Ltd., Plymouth Marine Laboratory, UK).

5.6 SAMPLE TESTING RESULTS

The historical perchlorate levels at the locations from which groundwater was collected, and the conductivity and pH of groundwater samples used for the project are listed in Table 5-3.

Data from the groundwater testing by benchtop bioassay, reference method performed at a commercial laboratory, and IC testing in the PI's laboratory at UCB, are listed in Appendices B, C, and D, respectively. The comparison of results from the three methods, and the accuracy of the benchtop bioassay and UCB IC analysis compared to the results from the reference method performed in a commercial laboratory are listed in Table 5-4. The accuracy of the benchtop bioassay was considered acceptable when the percent difference (PD) from the reference test was 30 % or less. The 30 % value for the acceptability metric was adopted based on a consideration of previous results with the plate reader version of the bioassay, reported by Heinnickel et al.[2]. A t-test analysis of results obtained by the bioassay and by an IC reference method, indicated that the methods were not significantly different ($\alpha = 0.05$) for three different groundwater samples. PD values for the bioassay compared to the IC reference method were 14, 19, and 25 %.

Table 5-3. Conductivity, pH, and Historical Perchlorate of Groundwater Sources

Site	Location	Historical perchlorate (µg/L)	Conductivity (µS/cm)	pH	Volume collected (gallons)
102, Hill AFB	U9-16-007	37	653	8.3	3
103, McGregor NWIRP	OFFWS-37	50	616	8.0	2
104, JPL	MW-16	<1	589	7.2	3
105, Fontana Water Co.	F-17C	8	396	7.5	3
106, Massachusetts Military Reservation	J-3 INF	5.8	45	6.5	3
107, LLNL	W-854-1823	16	968	8.0	3

As noted in Table 5-4, benchtop bioassay results were acceptable for the Fontana Water Co. groundwater (i.e. PD was 18 %) and boarderline with Hill AFB groundwater (i.e. PD was 32 %). The JPL source was collected from a location, which historically has had no detectable perchlorate (Table 5-3), in order to evaluate the potential for false positive results with the benchtop bioassay. The accuracy of the benchtop bioassay with JPL groundwater was considered qualitatively acceptable since the standard deviation for replicate SPE eluate sets was small (i.e. ± 2 µg/L), and the estimated perchlorate was low (i.e. 3 µg/L). It was not possible to make a quantitative PD calculation because the perchlorate by reference methods was <MRL.

The accuracy of the benchtop bioassay was unacceptable for McGregor, MMR and LLNL sources with PD calculated as 325, 429, and 92 %, respectively. The average for the McGregor and MMR benchtop bioassay results were 4 to 5 times higher than the corresponding results with the reference method performed in a commercial laboratory. In addition there was pronounced lack of agreement in perchlorate estimates among the replicate SPE eluate sets for McGregor (range 43 to 720 µg/L) and MMR (range -3 to 83 µg/L). The r^2 values for the different SAM regression lines were also variable for these two sources, ranging from 0.002 to 0.627 for McGregor, and from 0.472 to 0.998 for MMR. By contrast, the average for LLNL benchtop bioassay results was 13 times lower than the reference method performed in a commercial laboratory, there was good agreement among replicate SPE eluate sets (i.e. the standard deviation of the mean was ± 3 µg/L), and the r^2 values for the different SAM regression lines ranged from 0.953 to 0.999. Thus the benchtop bioassay failed for McGregor and MMR, probably due at least in part to inadequate reduction of the reaction mixtures prior to adding cell extract. However, with the LLNL source, there seemed to be interference with the benchtop bioassay.

Detailed data from benchtop bioassay testing are archived in Appendix B where Table B-1 summarizes for each eluate set the perchlorate concentration determined from standard additions method (SAM) regression lines ± the error of the SAM x-intercept. The averages from all replicates of a source ± standard deviation are also noted in Table B-1 and repeated in Table 5-4. Representative results from SAM regression lines are shown in Figure 5-1. The data shown are a subset of data from Appendix B (i.e. drawn from Figures B-102, 103, 104, 105, 106, and 107). The variability in x-intercepts seen for the McGregor and MMR panels contrast with the much less variable results seen in the panels for the other groundwater sources.

Each batch of benchtop bioassays included a negative control (i.e. no perchlorate added to the reaction mixture) and a 40 µM perchlorate positive control. Usually a 20 µM perchlorate positive control was also included. The negative and positive controls were tested in enzyme buffer,

which contained MOPS and NaCl, pH 7.2, but had not been exposed to SPE cartridges. In all instances, these controls performed in a consistent manner, showing that perchlorate reductase had the expected level of activity. The average x-intercept error was low, ± 1.6 µM perchlorate (n = 15), see Appendix L, Table L-2.

Table 5-4. Perchlorate Concentration Estimated by Different Methods and Accuracy Evaluated Using Percent Difference Comparisons[a]

Source	Method	Perchlorate concentration (µg/L)									Average ± SD	PD[b] (%)	Benchtop bioassay accuracy[c]
		A replicates			B replicates			C replicates					
		1	2	3	1	2	3	1	2	3			
102, Hill AFB	IC Ref. test	36	40	32	47	44	37	33	35	38	38±5	0	boarderline
	IC, UCB	34	35	35	35	35	36	36	36	36	35±1	8	
	Benchtop	23	21	14	20	23	22	31	45	33	26±9	32	
103, McGre-gor NWIRP	IC Ref. test	59	60	61	98	56	55	NA	NA	NA	65±16	0	unacceptable
	IC, UCB	58	58	59	54	55	55	NA	NA	NA	57±2	12	
	Benchtop	43	106	673	58	720	55	NA	NA	NA	276±327	325	
104, JPL	IC Ref. test	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	NA	qualitatively acceptable
	IC, UCB	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	NA	
	Benchtop	3	2	5	0	4	5	4	2	1	3±2	NA	
105, Fontana Water Co.	LC/MS Ref.	11	11	11	11	11	11	9.3	9.8	9.5	11±1	0	acceptable
	IC, UCB	11	11	11	11	11	11	11	10	10	11±0.4	0	
	Benchtop	15	8	11	5	8	5	7	9	10	9±3	18	
106, MMR	LC/MS Ref.	5.5	5.3	4.9	5.2	5.3	5.3	4.9	4.9	4.8	5.1±0.3	0	unacceptable
	IC, UCB	6	6	6	6	7	6	6	6	6	6±0.3	18	
	Benchtop	-3	23	51	36	23	9	83	7	18	27±26	429	
107, LLNL	LC/MS Ref.	14	14	13	15	14	13	12	13	12	13±1	0	unacceptable
	IC, UCB	16	16	16	15	15	15	16	16	16	16±1	23	
	Benchtop	2	-3	-4	6	1	0	1	1	2	1±3	92	

[a]NA denotes not applicable, only two gallons of groundwater were obtained for testing source 103, and PD couldn't be calculated for source 104
[b]Percent difference (PD) compared to the results from the reference test performed in a commercial laboratory. PD was calculated as the absolute value (of the average reference test outcome minus the average from testing by another method) ÷ (the average reference test outcome) and expressed as a percent
[c]Bioassay accuracy was considered acceptable when the PD was 30% or less

The data for Hill AFB and Fontana Water Co. (judged to have boarderline and acceptable accuracy, respectively, as shown in Table 5-4), were further analyzed by a one-way ANOVA to examine the data for differences among the methods. The outcomes are listed in Table 5-5. Grouping among the different methods was evaluated by the Tukey Method using 95% simultaneous confidence intervals. This analysis indicated that the methods were significantly different for the Hill AFB source and also that the benchtop bioassay was the cause of this difference. The methods were not significantly different for the Fontana Water Co. groundwater.

Table 5-5. ANOVA for Reference Method Performed by a Commercial Laboratory, IC at UCB, and the Benchtop Bioassay, and Grouping Using the Tukey Method

Source	Average perchlorate results ± SD (µg/L)			F	ANOVA P-value	Tests are significantly different (α = 0.05)
	Reference test	IC at UCB	Benchtop bioassay			
102, Hill AFB	38.000 ± 4.950	35.33 ± 0.707	25.778 ± 9.176	10.21	0.001	yes
105, Fontana Water Co.	10.511 ± 0.744	10.778 ± 0.441	8.778 ± 3.383	2.61	0.094	no
Grouping by Tukey Method						
102, Hill AFB	Group 1	Group 1	Group 2	Benchtop bioassay different from reference analytical methods		
105, Fontana Water Co.	NA	NA	NA	Methods are not different from one another, Tukey Method not applied		

In summary, the benchtop bioassay in its current form is not suitable as a screening test for perchlorate in groundwater. Six groundwater sources were tested. Results compared to the reference analytical method performed in a commercial laboratory were quantitatively acceptable for the Fontana Water Co. source, and qualitatively acceptable for the JPL source. The benchtop bioassay for the Hill AFB sample was significantly different from the reference method by ANOVA analysis. The benchtop bioassay exhibited characteristics of a failed test for McGregor and MMR sources, in that replicates were highly variable and the average estimate for perchlorate concentration was much higher than for the reference analytical method. Finally, for the LLNL source, there appeared to be interference with the benchtop bioassay since the average estimate for perchlorate concentration was much lower than for the reference analytical method.

5.7   TESTING FOR FACTORS THAT MAY IMPACT BIOASSAY PERFORMANCE

Testing was done to investigate factors that could impact bioassay performance. This included: 1. assessing the adequacy of the SPE step in concentrating perchlorate from groundwater and confirming that nitrate would not be extracted along with perchlorate, 2. determining whether SPE eluates of groundwater tested by the plate reader bioassay format – which is performed in an anaerobic chamber – met accuracy criteria, 3. characterizing groundwater samples for other anions by IC, and 4. evaluating the profile of elements in groundwater sources and corresponding SPE eluates by ICPMS.

Figure B-102-batch1. Hill AFB SAM sets A1, A2, A3, benchtop bioassay with negative control subtracted, 33 uL CKB 8/3/2011 xt, tested 11Aug2011

Figure B-103-batch1. NWIRP McGregor SAM sets A1, A2, A3, benchtop bioassay with negative control subtracted, 33 uL CKB 8/3/2011 xt, tested 6Nov2011

Figure B-104-batch2. JPL SAM sets B1, B2, B3, benchtop bioassay with negative control subtracted, 33 uL CKB 8/3/2011 xt, tested 12Dec2011

Figure 5-1. Representative standard additions method (SAM) regression lines from benchtop bioassay testing of the six groundwater sources analyzed in the project
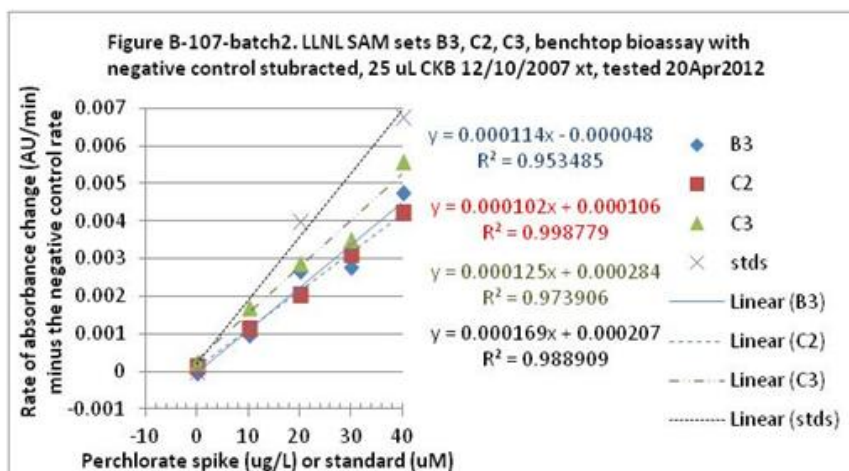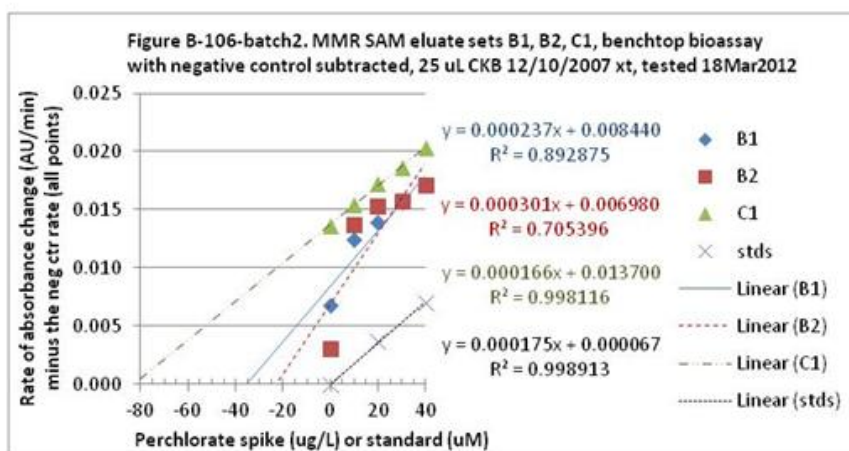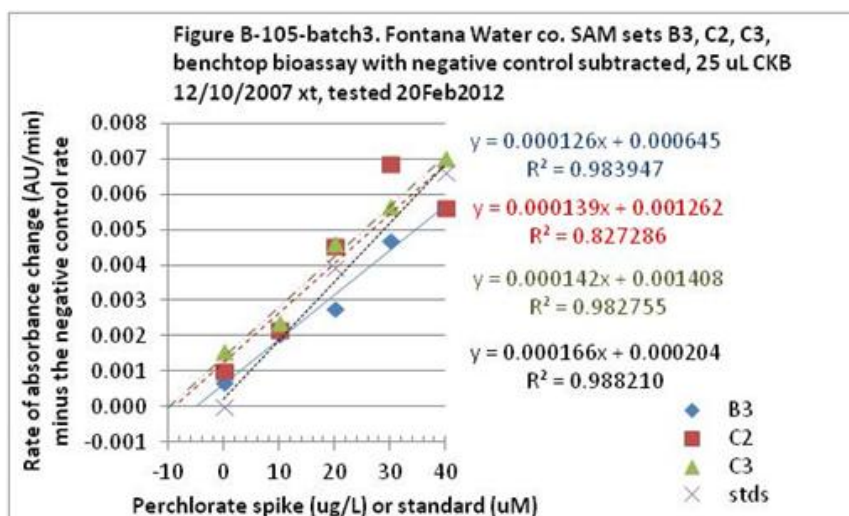
Figure 5-1, continued. Representative standard additions method (SAM) regression lines from benchtop bioassay testing of the six groundwater sources analyzed in the project

As discussed in more detail below, these studies indicated the following. 1. The SPE step performed as expected to concentrate perchlorate. 2. The accuracy of the anaerobic plate reader bioassay was acceptable for the five groundwater sources that could be evaluated quantitatively. Apparently the interference phenomenon observed when LLNL SPE eluates were tested by the benchtop bioassay was not seen when testing was repeated in the anaerobic chamber using the plate reader bioassay format. 3. IC analysis for common anions showed that nitrate was present in SPE effluents at about the same concentration as in the corresponding groundwater. In addition, about 9 mg/L of bromide was present in SPE effluents, much more than in the groundwater sources. The probable source of this bromide was the DTAB used to condition cartridges. 4. ICPMS analysis of groundwater and corresponding alkaline MOPS-NaCl SPE eluants showed that uranium is extracted by the SPE step and recovered with good efficiency in SPE eluates. In addition, the amount of bromine present in SPE eluates was much greater than in corresponding groundwater sources or the alkaline MOPS-NaCl solution used to elute cartridges. Again, the probable source of the high bromine in eluates is the DTAB used to condition and rinse cartridges. Eluates prepared from LLNL groundwater had the highest uranium concentration, about 9 µM. Since the presence of uranium might be correlated with the interference of the benchtop bioassay seen with the LLNL source, uranium was tested for possible interference with perchlorate reductase activity by adding it directly to benchtop bioassay reaction mixtures. However, no interference was observed when 1 to 10 µM uranium was present in reaction mixtures in addition to the enzyme substrate, perchlorate (40 µM).

5.7.1 Assessment of the Adequacy of the SPE Step

Several approaches were used to confirm that the SPE step met expectations. One was to troubleshoot the unacceptable accuracy performance of the benchtop bioassay with MMR and LLNL groundwater by using IC to reanalyze several SPE eluate sets for perchlorate. Results are archived in Appendix G. Perchlorate concentrations were determined by IC and then plotted by the standard additions method (SAM) to estimate the concentration of perchlorate in groundwater from the x-intercept of the SAM regression line. Three eluate sets were tested in this way for MMR groundwater, and the estimated perchlorate was in good agreement with the reference analytical method for each of the sets tested (see Appendix G, Table G-1). Two eluate sets were tested for LLNL groundwater. The estimated perchlorate was in good agreement with the reference analytical method for the C3 set, but significantly higher for B1 set (see Table G-1). Nevertheless, it is clear that the apparent interference observed with benchtop bioassay testing of LLNL groundwater was not due to failure of the SPE step with respect to the recovery of perchlorate in eluates.

Another approach was to analyze the SPE effluent after groundwater sources were applied to cartridges by the standard protocol (see Appendix H). The amount of nitrate was similar in effluents and groundwater sources. Thus nitrate was not extracted by the SPE step, which thereby effectively separated perchlorate and nitrate. Chlorate is the other anion that could compete with perchlorate as a substrate for perchlorate reductase. However, the groundwater sources included in this study did not contain levels of chlorate that were of concern.

5.7.2   Plate Reader Bioassay Data

Three to five SPE eluate sets for different groundwater sources, which had previously been tested by the benchtop bioassay, were retested using the anaerobic plate reader bioassay. The data are archived in Appendix F. Table 5-6 shows the testing results and accuracy assessment for the plate reader format of the bioassay. The accuracy of the plate reader bioassay was considered acceptable when the percent difference (PD) from the reference analytical method, using the same reference values as are listed in Table 5-4, was 30 % or less.
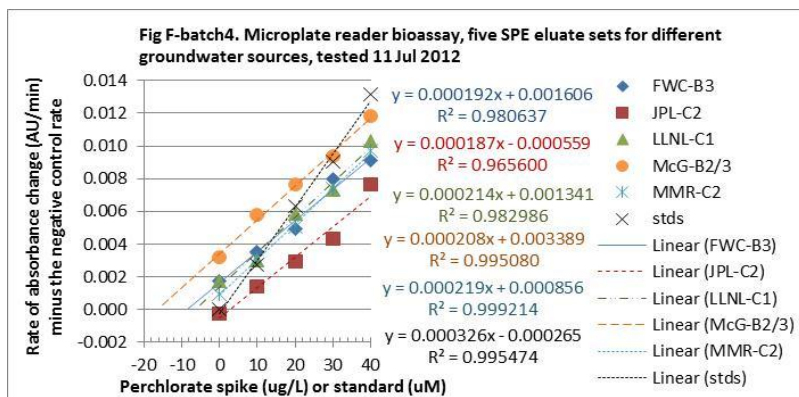
Figure 5-2. Perchlorate anaerobic plate reader bioassay results for batch 4

Figure 5-2 shows representa-tive results from the anaerobic plate reader bioassay testing. Pictured is a subset of data archived in Appendix F (i.e. Figure F-4) for plate reader bioassay batch 4. The standard additions method regression lines have high r^2 values for all the sources tested. Accuracy was acceptable with the five groundwater sources amenable to quantitative comp-arisons among the methods (Table 5-6). It was considered only qualitatively acceptable for the JPL source, since the estimated perchlorate by the plate reader bioassay was low and the standard deviation of replicate sets was within the range seen for other groundwater sources (i.e. ± 2 to 7 µg/L, see Table 5-6). A quantitative comparison wasn't possible for the JPL source since the reference method results were not quantitative (i.e. <MRL). Table 5-7 shows ANOVA testing for the plate reader bioassay compared to the reference analytical methods tested in a commercial laboratory and IC at UCB (same data as listed in Table 5-4). The three methods were not significantly different for Hill AFB, McGregor NWIRP, and Fontana Water Co. at the 5 % level (i.e. α = 0.05). Although the three tests were significantly different by ANOVA for MMR, grouping by the Tukey Method showed that the three methods grouped together. Thus it seems the assessment of differences among the methods by ANOVA should not overshadow the fact that the accuracy of the plate reader bioassay was acceptable (Table 5-6) as was the IC at UCB accuracy (Table 5-4) relative to the reference method at a commercial laboratory. Finally, ANOVA showed that for LLNL groundwater the three methods were different. Grouping by the Tukey Method showed that the anaerobic plate reader bioassay and the reference method grouped together while the IC at UCB method was the cause of the ANOVA difference.

Each batch of anaerobic plate reader bioassays included at least one negative control well (i.e. no perchlorate added to the reaction mixture). The rate of 340 nm absorbance change/minute for the negative control was subtracted from the rate of NADH oxidation for all the other reaction mixtures (Appendix F, Tables F-2 though F-6). For batches 1, 4, and 5 perchlorate standards were also tested at 10, 20, 30, and 40 µM concentrations. Negative and positive controls were tested in enzyme buffer as previously described for benchtop bioassay controls. Regression lines

Table 5-6. Perchlorate Concentration Estimated by Plate Reader Bioassay and Accuracy Evaluated Using the Percent Difference Compared to the Reference Analytical Method[a]

Source	Method	Perchlorate concentration (µg/L)									Average ± SD	PD[b] (%)	Plate reader bioassay accuracy[c]
		A replicates			B replicates			C replicates					
		1	2	3	1	2	3	1	2	3			
102, Hill AFB	PR assay	36	43, 27[d]	23	38	nt	nt	nt	nt	34	34±7	11	acceptable
103, McGregor NWIRP	PR assay	nt	53	45	nt	49	nt	NA	NA	NA	49±4	25	acceptable
104, JPL	PR assay	nt	nt	nt	-10	nt	2	nt	-3	nt	-4±6	NA	qualitatively acceptable
105, Fontana Water Co.	PR assay	19	11	nt	nt	nt	8	nt	nt	10	12±5	9	acceptable
106, MMR	PR assay	nt	3	4	8	nt	nt	nt	4	nt	4.8±2.2	6	acceptable
107, LLNL	PR assay	nt	nt	nt	13	14	nt	6	nt	nt	11±4	15	acceptable

[a]The "nt" denotes not tested, NA denotes not applicable

[b]Percent difference (PD) compared to the results from the reference test performed in a commercial laboratory. PD was calculated as the absolute value (of the average reference test outcome minus the average from testing by another method) ÷ (the average reference test outcome) and expressed as a percent

[c]Bioassay accuracy was considered acceptable when the PD was 30% or less

[d]The Hill AFB A2 SPE set was tested by the plate reader bioassay twice. See Appendix F

Table 5-7. ANOVA for Reference Method Performed by a Commercial Laboratory, IC at UCB, and the Plate Reader Bioassay, and Grouping Using the Tukey Method

Source	Average perchlorate results ± SD (µg/L)			F	ANOVA P-value	Tests are significantly different (α = 0.05)
	Reference test	IC at UCB	Plate reader bioassay			
102, Hill AFB	38.00 ±4.950	35.33 ±0.707	33.500±7.342	1.72	0.203	no
103, McGregor NWIRP	64.83±16.41	56.50±2.07	49.00±4.00	2.29	0.144	no
103, JPL	<2	<4	-3.667 ± 6.028	NA	NA	qualitatively not different
104, Fontana Water Co.	10.511 ±0.744	10.778±0.441	12.000±4.830	0.79	0.467	no
106, MMR	5.122±0.249	6.111±0.333	4.750±2.217	4.04	0.035	yes
107, LLNL	13.333±1.000	15.667±0.500	11.000 ± 4.359	10.50	0.001	yes
Grouping by Tukey Method[a]						
102, Hill AFB	NA	NA	NA	NA		
103, McGregor NWIRP	NA	NA	NA	NA		
103, JPL	NA	NA	NA	NA		
104, Fontana Water Co.	NA	NA	NA	NA		
106, MMR	Group 1	Group 1	Group 1	Methods not different (group together)		
107, LLNL	Group 1	Group 2	Group 1	Plate reader bioassay and reference test are not different. IC at UCB is the cause of the difference by ANOVA analysis		

[a]Grouping by the Tukey Method is only performed when the p-value from ANOVA analysis is < 0.05

for plots of the rate of reaction vs. concentration for perchlorate standards intersected the x-axis close to the origin and showed low x-intercept errors (i.e. ± 0.64 µM, n = 3). See Appendix L, Table L-3.

Results with the anaerobic plate reader bioassay support the idea that this format of the bioassay could be an effective laboratory screening test for perchlorate[2]. A possible reservation regarding this suggestion is that generally the plate reader bioassay testing was done after considerable time had passed. Although perchlorate is stable in the environment, ageing of SPE eluates may have made them more amenable to analysis by bioassay methods. It is interesting to note, however, that Hill AFB SPE eluate sets were analyzed on two occasions. The first preceded benchtop bioassay testing, and the second was 11 months later. The average estimate of perchlorate concentration was similar for the two batches (Appendix F, Table F-1), suggesting that for Hill SPE eluate sets, ageing had little impact on results obtained by the anaerobic plate reader bioassay. Additional testing would be needed to resolve this point.

5.7.3 OTHER ANIONS IN GROUNDWATER SOURCES

IC analysis was used to determine levels of common anions in the groundwater sources, and the data are listed in Tables H-1 and H-2 in Appendix H. The results showed that the levels of major anions in groundwater sources were not remarkable. Chlorate, which is an alternative substrate for perchlorate reductase, was not present in groundwater sources. Nitrate was not extracted by SPE cartridges. Bromide was much higher in SPE effluents than in groundwater sources and must have come from residual DTAB present in cartridges after conditioning.

5.7.4 ELEMENT PROFILES IN GROUNDWATER SOURCES AND SPE ELUATES

Groundwater sources and freshly prepared SPE eluates were analyzed by ICPMS. Results are listed in Appendix I, Table I-1 for groundwater, and Table I-2 for corresponding SPE eluates. Bromine was present at much higher amounts in SPE eluates than in corresponding groundwater sources or the alkaline MOPS-NaCl solution used to elute perchlorate from cartridges. Non-metric multi-dimentional scaling (MDS) analysis of the data on elements from Tables I-1 and I-2 indicated that overall patterns were different for groundwater and SPE eluates. The MDS analysis facilitated an assessment of which elements were more concentrated in SPE eluates than in the corre-sponding groundwater source. As discussed in more depth in Appendix I, uranium was

Figure 5-3. MDS plot based on ICPMS data for groundwater and SPE eluates with superimposed circles representing the concentration of uranium

concentrated in eluates, and was highest in the LLNL eluate. See Figure 5-3, which shows the panel for uranium from Appendix I, Figure I-4. Relationships among groundwater sources grouped on the left-hand side of the plot, and are identified with the prefix "G". Relationships among SPE eluates grouped on the right-hand side of the plot, and are identified with the suffix "-DI". Superimposed on the MDS plot are circles representing the concentration of uranium. In addition, ICPMS analysis was done on some of the SPE eluate sets from LLNL, Fontana Water Co., and Hill AFB that had previously been tested by the benchtop bioassay (Table I-5). This confirmed that the amount of uranium present in eluates analyzed by bioassay testing was similar to that seen in freshly prepared SPE eluates.

The ICPMS data seemed consistent with the possibility that the presence of fairly high levels of uranium in LLNL SPE eluates might have impacted perchlorate reductase activity in the benchtop bioassay testing.

5.7.5 TESTING URANIUM(VI) FOR INTERFERENCE WITH PERCHLORATE REDUCTASE ACTIVITY

Testing was done to determine whether adding uranium (VI) directly to reaction mixtures interfered with the benchtop bioassay for perchlorate (Appendix J). Duplicate reactions containing 40 µM perchlorate and 0, 1, 2, 3, 5, 8, or 10 µM uranium were tested. All reaction mixtures had the same perchlorate reductase activity, showing that the addition of uranium had no effect on enzyme activity.

6.0 PERFORMANCE ASSESSMENT

6.1 QUANTITATIVE PERFORMANCE OBJECTIVE 1: COMPARE BENCHTOP BIOASSAY RESULTS TO REFERENCE ANALYTICAL METHOD RESULTS

The percent difference between benchtop bioassay results and the reference method performed in a commercial laboratory was the metric used to evaluate the accuracy of the bioassay method. By the PD comparison, the accuracy of the benchtop bioassay was acceptable for Fontana Water Co. and boarderline for Hill AFB (Table 5-4). Results with JPL couldn't be compared quantitatively since the perchlorate from this source was <MRL for the reference method. The benchtop bioassay test failed for McGregor NWIRP, Massachusetts Military Reservation (MMR), and Lawrence Livermore National Laboratory (LLNL) groundwater sources, i.e. the PD values were 325, 429, and 92 %, respectively. In most cases, the variation among replicates was greater for the benchtop bioassay than for the other analytical methods. The lack of agreement was pronounced for the McGregor and MMR testing where relative standard deviations (i.e. the standard deviation ÷ average, expressed as a percent) were 118 and 96 %, respectively. However, for LLNL testing there was good agreement among replicate SPE eluate sets, suggesting there may have been interference with the bioassay for this source.

ANOVA was performed using the Hill AFB and Fontana Water Co. data (see Table 5-5). This analysis showed that the three methods were not significantly different for Fontana Water Co. groundwater. However, the benchtop bioassay was significantly different from the analytical methods for the Hill AFB source.

6.2 QUANTITATIVE PERFORMANCE OBJECTIVE 2: COMPARE BENCHTOP BIOASSAY FIELD KIT RESULTS TO SITE'S REFERENCE METHOD RESULTS

Results with the benchtop bioassay did not justify the development of a field kit, hence no testing was done relative to this objective.

6.3 QUALITATIVE PERFORMANCE OBJECTIVE: FIELD KIT EASE OF USE ASSESSMENT

No field kit was developed, hence no assessment was done relative to evaluate this objective.

7.0   COST ASSESSMENT

A costs assessment for implementing a field screening test for perchlorate based on the benchtop bioassay format is not applicable at this point since the current version of the assay is not suitable for this use.

8.0   IMPLEMENTATION ISSUES

Implementation issues are not relevant at this point since the current version of the benchtop bioassay is not suitable for use as a screening test for perchlorate in the field. The accuracy of the benchtop bioassay with different groundwater sources was evaluated by comparison to results from the reference analytical method performed in a commercial laboratory. The metric used to determine whether the bioassay level of accuracy was acceptable was the percent difference. The criterion for acceptable accuracy was a percent difference (PD) of 30 % or less. The reference analytical methods used were either LC/MS tested according to EPA Method 6850, or IC tested according to EPA Method 314.0. In general, the benchtop assay performed poorly. While the accuracy was qualatively acceptable for the JPL source and quantitatively acceptable for the Fontana Water Co. source, it was only boarderline for the Hill AFB source and was not acceptable (i.e > 30 % PD) for the three other sources: NWIRP McGregor, Massachusetts Military Reservation (MMR), and LLNL. There was a pronounced lack of agreement among replicates tested in the benchtop bioassay for NWIRP McGregor and Massachusetts Military Reservation sources.

Retesting of the water samples with the bioassay in an anaerobic chamber with a nitrogen/hydrogen atmosphere provided a different outcome. In this instance the accuracy was acceptable for all five groundwater eluates amenable to quantitative testing: Hill AFB, NWIRP McGregor, Fontana Water Co., Massachusetts Military Reservation, and LLNL. Furthermore, ANOVA and Tukey Method statistical analyses indicated that the anaerobic bioassay and the reference method in a commercial laboratory were not significantly different. This finding pointed to oxygen interference in the benchtop assay as the culprit of inaccuracy. In support of this, creating sufficiently reduced conditions in reaction mixtures by the non-enzymatic reaction between NADH and PMS proved unreliable. The non-enzymatic oxidation of NADH in the presence of PMS was unpredictable and far greater for the benchtop format than observed when reactions were performed in the anaerobic chamber. NADH oxidation seen during the preincubation period was even variable and unpredictable among replicate SPE eluate sets (see Appendix L, Table L-1) and often of much greater magnitude than occurs during perchlorate reduction by perchlorate reductase.

While consideration was given to adding reductants in addition to the NADH and PMS system, to more chemically remove oxygen from the SPE eluates prior to bioassay analysis, this approach was dismissed because of possible interference. As a point in case, mixing of the reductant cysteine (an amino acid with a sulfhydral group) with oxidized PMS resulted in the rapid formation of a red degradation product, showing that cysteine wouldn't be suitable as an additional reductant for the benchtop bioassay. Other reductants such as ascorbic acid, dithiothreitol, or dithionite were not attractive, since these reduce PMS[2] directly, and would interfere with the stoichiometry of NADH and perchlorate in the bioassay. Thus the only approach that could be adopted for benchtop bioassay was to extend the preincubation period during which residual oxygen is removed non-enzymatically and amend reaction mixtures with additional NADH prior to addition of extract to start the enzymatic reaction. However, this approach was not sufficiently effective or reproducible as a means of making reaction mixtures suitably reduced prior to perchlorate reductase addition creating unpredictability in the bioassay for perchlorate determination.

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APPENDICES

Appendix A: POINTS OF CONTACT

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Appendix B: BENCHTOP BIOASSAY DATA

Table B-1 summarizes the results from benchtop bioassay testing of SPE eluate sets using the standard additions method to estimate the perchlorate concentration in groundwater sources.

Table B-1. Perchlorate determined by benchtop bioassay in SPE eluate sets[a]

Site	Para-meter[b]	A1	A2	A3	B1	B2	B3	C1	C2	C3	Average ± SD
102, Hill AFB	SPE date	27 Jul 2011	27 Jul 2011	27 Jul 2011	6 Aug 2011	7 Aug 2011	9 Aug 2011	7 Aug 2011	7 Aug 2011	9 Aug 2011	
	Batch	1	1	1	2	3	2	3	3	2	
	μg/L	23±3	21±3	14±6	20±8	23±4	22±3	31±4	45±16	33±6	26±9
103, McGre-gor NWIRP[c]	SPE date	5 Nov 2011	5 Nov 2011	5Nov 2011	7 Nov 2011	8 Nov 2011	8 Nov 2011	NA	NA	NA	
	Batch	1	1	1	2	2	2				
	μg/L	43±36	106 ±210	673 ±8574	58±68	720 ±3870	55±41				276±327
104, JPL	SPE date	8 Dec 2011	8 Dec 2011	8 Dec 2011	11 Dec 2011	11 Dec 2011	11 Dec 2011	11 Dec 2011	11 Dec 2011	13 Dec 2011	
	Batch	3	1	1	2	2	2	3	3	4	
	μg/L	3±2	2±9	5±3	0±5	4±1	5±3	4±3	2±4	1±1	3±2
105, Fontana Water Co.	SPE date	13 Feb 2012	13 Feb 2012	13 Feb 2012	14 Feb 2012	15 Feb 2012	15 Feb 2012	17 Feb 2012	17 Feb 2012	17 Feb 2012	
	Batch	1	1	1	2	2	3	2	3	3	
	μg/L	15±4	8±0	11±3	5±3	8±3	5±2	7±2	9±7	10±2	9±3
106, MMR	SPE date	9 Mar 2012	12 Mar 2012	12 Mar 2012	13 Mar 2012	13 Mar 2012	13 Mar 2012	14 Mar 2012	14 Mar 2012	14 Mar 2012	
	Batch	1	1	1	2	2	3	2	3	3	
	μg/L	-3±5	23±4	51±34	36±9	23±13	9±9	83±2	7±3	18±10	27±26
107, LLNL	SPE date	6 Apr 2012	6 Apr 2012	9 Apr 2012	12 Apr 2012	16 Apr 2012	16 Apr 2012	17 Apr 2012	17 Apr 2012	17 Apr 2012	
	Batch	3	3	3	1	1	2	1	2	2	
	μg/L	2±0	-3±2	-4±1	6±2	1±2	0±3	1±2	1±0	2±2	1±3

[a]The x-intercept error from the standard additions method regression line is listed with individual perchlorate estimates. The overall average is also listed for each groundwater source ± the standard deviation. NA denotes not applicable
[b]"SPE date" corresponds to the preparation date for an SPE eluate set. The testing batch that included a specific eluate set is listed in the table, and the date on which batches were tested is listed in the text below.
[c]Only two full gallon samples (bottles A and B) were available from the McGregor groundwater source.

Testing was performed in batches and as many as 18 perchlorate reductase reactions in individual cuvettes were tested at a time. Batches were tested on the following dates:
Hill AFB: batch *1* (11 Aug 2011), batch *2* (11 Aug 2011), batch *3* (12 Aug 2011)
McGregore: batch *1* (6 Nov 2011), batch *2* (9 Nov 2011)
Jet Propulsion Lab: batch *1* (9 Dec 2011), batch *2* (12 Dec 2011), batch *3* (12 Dec 2011), batch *4* (16 Dec 2011)
Fontana Water Company: batch *1* (16 Feb 2012), batch *2* (18 Feb 2012), batch *3* (20 Feb 2012)
Massachusetts Military Reservation: batch *1* (15 Mar 2012), batch *2* (18 Mar 2012), batch *3* (19 Mar 2012)
Lawrence Livermore National Laboratory: batch *1* (20 Apr 2012), batch *2* (23 Apr 2012), batch *3* (24 Apr 2012).

The bioassay rates (i.e. 340 nm absorbance change/minute) used in the standard additions method to determine the perchlorate (µg/L) for SPE eluate sets tested in the different batches of a groundwater source are listed in Tables B-102 (Hill AFB), B-103 (NWIRP McGregor), B-104 (Jet Propulsion Laboratory), B-105 (Fontana Water Co.), B-106 (Massachusetts Military Reservation), and B-107 (Lawence Livermore National Laboratory). The perchlorate spikes added prior to applying samples to SPE cartridges were 0, 10, 20, 30, or 40 µg/L. Each table has an accompanying figure, which shows the standard additions method regression lines for the SPE eluate sets in a particular batch, from which perchlorate concentrations were determined. The Figures are designated as B-102 (Hill AFB), B-103 (NWIRP McGregor), B-104 (Jet Propulsion Laboratory), B-105 (Fontana Water Co.), B-106 (Massachusetts Military Reservation), and B-107 (Lawence Livermore National Laboratory).

Detailed experimental data, i.e. the 340 nm absorbance over time for SPE eluates, are presented last in Appendix B in a series of figures and tables for each batch tested. These show the NADH oxidation during preincubation periods as well as after the addition of extract having perchlorate reductase activity, which constitutes the initial time for bioassay reactions.

Groundwater sample 102, Hill AFB, location U9-16-007, collected 14Jul2011

Table B-102. Perchlorate benchtop bioassay analysis of standard additions method sets for Hill AFB groundwater collected 14 Jul 2011 from location U9-16-007[a]

Perchlorate Spike (µg/L)	Batch 1 (tested 11 Aug 2011)			Batch 2 (tested 11 Aug 2011)		
	A1	A2	A3	B1	B3	C3
0	0.0022	0.0022	0.0013	0.0023	0.0026	0.0045
10	0.0035	0.0033	0.0028	0.0047	0.0042	0.0045
20	0.0040	0.0037	0.0024	0.0038	0.0046	0.0063
30	0.0056	0.0051	0.0047	0.0058	0.0066	0.0076
40	0.0062	0.0064	0.0051	0.0078	0.0074	0.0089
Regression parameters for each set[b]						
y-intercept ± error	0.002280 ±0.000219	0.002100 ±0.00024	0.001360 ±0.000495	0.002460 ±0.000719	0.002680 ±0.000299	0.003980 ±0.000387
Slope ± error	0.000101 ±0.0000089	0.000102 ±0.0000098	0.000095 ±0.000020	0.000121 ±0.000029	0.000120 ±0.000012	0.000119 ±0.000016
r^2	0.9771	0.9731	0.8803	0.8498	0.9698	0.9496
x-intercept ± error	-23±3	-21±3	-14±6	-20±8	-22±3	-33±6
ClO_4^- in groundwater ± error (µg/L) [c]	23±3	21±3	14±6	20±8	22±3	33±6

Perchlorate Spike (µg/L)	Batch 3 (tested 12 Aug 2011)			Perchlorate Standards			
	B2	C1	C2	µM	Batch 1	Batch 2	Batch 3
0	0.0032	0.0039	0.0033	0	0.0000	0.0000	0.0000
10	0.0036	0.0045	0.0044	10	ND	ND	ND
20	0.0048	0.0055	0.0062	20	ND	ND	ND
30	0.0069	0.0074	0.0069	30	ND	ND	ND
40	0.0077	0.0083	0.0062	40	0.0043	0.0054	0.0052
Regression parameters for each set[b]							
y-intercept ± error	0.002780 ±0.000380	0.003580 ±0.000296	0.003740 ±0.000640		NA	NA	NA
Slope ± error	0.000123 ±0.000016	0.000117 ±0.000012	0.000083 ±0.000026		0.000108	0.000135	0.000130
r^2	0.9544	0.9689	0.7706		NA	NA	NA
x-intercept ± error	-23±4	-31±4	-45±16		NA	NA	NA
ClO_4^- in groundwater ± error (µg/L) [c]	23±4	31±4	45±16		NA	NA	NA

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.0030 (batch 1), 0.0010 (batch 2), 0.0011 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 33 µL of CKB 8/3/2011 extract. ND denotes not determined, NA denotes not applicable
[b]Regression parameters are from the regression line for each set (see Figure B-102). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard
[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept
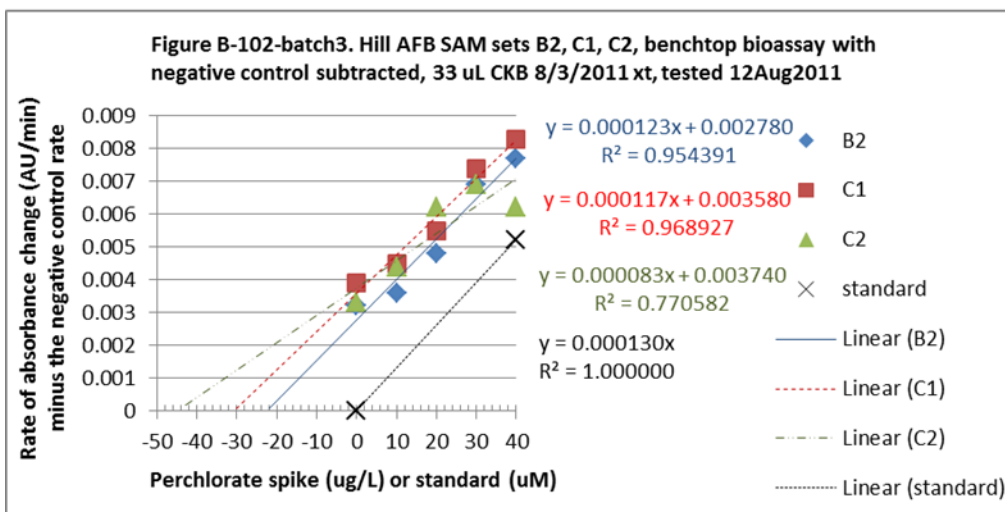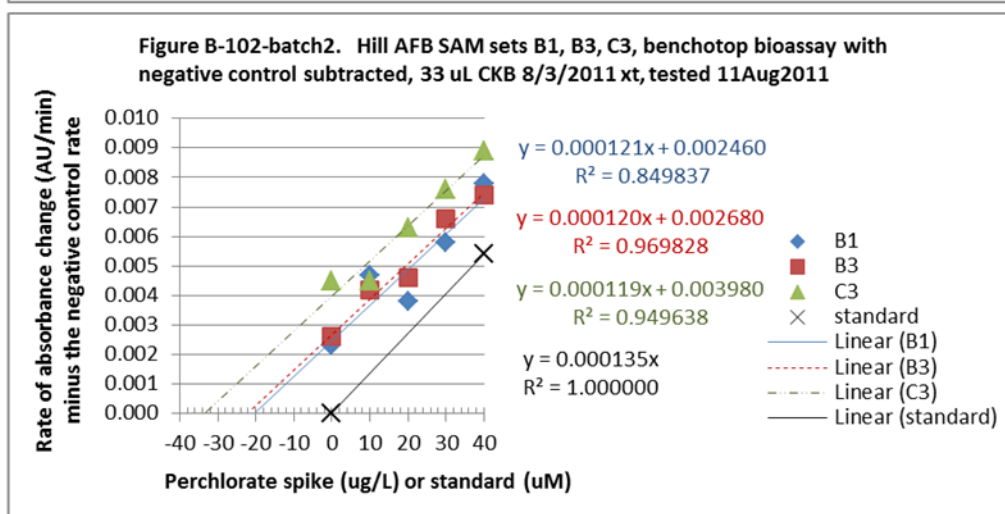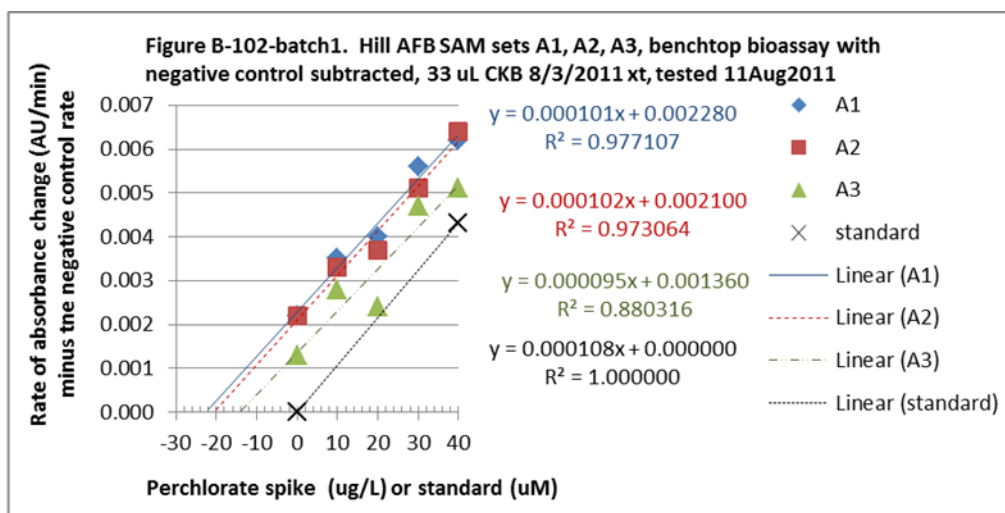
Figure B-102. Perchlorate benchtop bioassay analysis of standard additions method sets for Hill AFB groundwater collected 14 Jul 2011 from location U9-16-007 (See Table B-102)

Groundwater sample 103, NWIRP McGregor, location OFFWS-37, collected 13Oct2011

Table B-103. Perchlorate benchtop bioassay analysis of standard additions method sets for NWIRP McGregor groundwater collected 13 Oct 2011 from location OFFWS-37[a]

Perchlorate Spike (µg/L)	Batch 1 (tested 6 Nov 2011			Batch 2[d] (tested 9 Nov 2011)		
	A1	A2	A3	B1	B2	B3
0	0.0052	0.0127	0.0037	0.0062	0.0037	0.0011
10	0.0126	0.0055	0.0154	0.0028	0.0122	0.0122
20	0.0120	0.0174	0.0139	0.0035	0.0037	0.0096
30	0.0075	0.0076	0.0068	0.0053	0.0112	0.0136
40	0.0163	0.0164	0.0087	0.0127	0.0056	0.0135
Regression parameters for each set[b]						
y-intercept ± error	0.007300 ±0.003095	0.010020 ±0.004507	0.009420 ±0.004367	0.003000 ±0.002751	0.006720 ±0.003667	0.004760 ±0.002856
Slope ± error	0.000171 ±0.000126	0.000095 ±0.000184	0.000014 ±0.000178	0.000155 ±0.000112	0.000028 ±0.000150	0.000262 ±0.000117
r^2	0.3790	0.0816	0.0021	0.3884	0.0115	0.6273
x-intercept ± error	-43±36	-106±210	-673±8574	-19.4±22.6	-240.0±1290	-18.2±13.6
ClO_4^- in groundwater ± error (µg/L)[c]	43±36	106±210	673±8574	58±68	720±3870	55±41

Perchlorate Standards		
µM	Batch 1	Batch 2
0	0.0000	0.0000
10	ND	ND
20	0.0019	0.0025
30	ND	ND
40	0.0048	0.0042
Regression parameters for each set[b]		
y-intercept ± error	-0.000167 ±0.000373	0.000133 ±0.000298
Slope ± error	0.000120 ±0.000014	0.000105 ±0.000012
r^2	0.9857	0.9881
x-intercept ± error	1±3	-1±3

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.0022 (batch 1), 0.0018 (batch 2). Perchlorate reductase was added to cuvettes as the equivalent of 33 µL of CKB 8/3/2011 extract. ND denotes not determined

[b]Regression parameters are from the regression line for each set (see Figure B-103). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept.

[d]Groundwater tested in batch 2 was diluted with two parts deionized water prior to the addition of perchlorate spikes and application to SPE cartridges. Thus the estimate for perchlorate in groundwater for batch 2 was (-1) times the x-intercept times 3 (the dilution factor)

Figure B-103-batch1. NWIRP McGregor SAM sets A1, A2, A3, benchtop bioassay with negative control subtracted, 33 uL CKB 8/3/2011 xt, tested 6Nov2011

$y = 0.000171x + 0.007300$
$R^2 = 0.379025$

$y = 0.000095x + 0.010020$
$R^2 = 0.081609$

$y = 0.000014x + 0.009420$
$R^2 = 0.002051$

$y = 0.000120x - 0.000167$
$R^2 = 0.985739$

Figure B-103-batch2. NWIRP McGregor SAM sets B1, B2, B3, benchtop bioassay with negative contro subtracted, 33 uL CKB 8/3/2011 xt, tested 9Nov2011

$y = 0.000155x + 0.003000$
$R^2 = 0.388377$

$y = 0.000028x + 0.006720$
$R^2 = 0.011525$

$y = 0.000262x + 0.004760$
$R^2 = 0.627344$

$y = 0.000105x + 0.000133$
$R^2 = 0.988051$

Figure B-103. Perchlorate benchtop bioassay analysis of standard additions method sets for NWIRP McGregor groundwater collected 13 Oct 2011 from location OFFWS-37 (See Table B-103)

Groundwater sample 104, Jet Propulsion Laboratory (JPL), location MW-16, collected 14Nov2011

Table B-104. Perchlorate benchtop bioassay analysis of standard additions method sets for Jet Propulsion Laboratory groundwater collected 14 Nov 2011 from location MW-16[a]

Perchlorate Spike (µg/L)	Batch 1 (tested 9 Dec 2011)		Batch 2 (tested 12 Dec 2011)			Perchlorate Standards		
	A2	A3	B1	B2	B3	µM	Batch 1	Batch 2
0	0.0008	0.0009	0	0.0003	0.0006	0	0.0000	0.0000
10	0.0019	0.0017	0.0009	0.0013	0.0015	10	ND	ND
20	0.0025	0.0026	0.0025	0.0024	0.0027	20	0.0023	0.0021
30	0.0098	ND	0.0049	0.0032	0.0044	30	ND	ND
40	0.0069	0.0056	0.0041	0.0041	0.0046	40	0.0046	0.0049
Regression parameter for each set[b]								
y-intercept ± error	0.000360 ±0.001888	0.000620 ±0.000299	0.000040 ±0.000670	0.000360 ±0.000073	0.000580 ±0.000300		0.000000 ±0.000000	-0.000117 ±0.00026
Slope ± error	0.000201 ±0.000077	0.000119 ±0.000013	0.000122 ±0.000027	0.000095 ±0.000003	0.000109 ±0.000012		0.000115 ±0.000000	0.000123 ±0.000010
r^2	0.6938	0.9764	0.8690	0.9970	0.9634		1.0000	0.9932
x-intercept	-2±9	-5±3	-0±5	-4±1	-5±3		0±0	1±2
ClO_4^- in groundwater ± error (µg/L)[c]	2±9	5±3	0±5	4±1	5±3		NA	NA

Perchlorate Spike (µg/L)	Batch 3 (tested 12 Dec 2011)			Batch 4 (tested 16 Dec 2011)	Perchlorate Standards		
	A1	C1	C2	C3	µM	Batch 3	Batch 4
0	0.0004	0.0003	0.0004	nd	0	0.0000	0.0000
10	0.0008	0.0012	0.0013	0.0009	10	ND	ND
20	0.0023	0.003	0.002	0.0016	20	0.0028	0.0021
30	0.0031	0.003	0.0028	0.0025	30	ND	ND
40	0.0039	0.0045	0.0049	0.0033	40	0.0048	0.0047
Regression parameters for each set[b]							
y-intercept ± error	0.000240 ±0.000205	0.000360 ±0.000339	0.000180 ±0.000370	0.000050 ±0.000072		-0.000067 ±0.000149	-0.000083 ±0.000186
Slope ± error	0.000093 ±0.000008	0.000102 ±0.000014	0.000105 ±0.000015	0.000081 ±0.000003		0.000120 ±0.000006	0.000118 ±0.000007
r^2	0.9762	0.9475	0.9417	0.9979		0.9977	0.9962
x-intercept	-3±2	-4±3	-2±4	-1±1		1±1	1±2
ClO_4^- in groundwater ± error (µg/L)[c]	3±2	4±3	2±4	1±1		NA	NA

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. ND denotes not determined, NA denotes not applicable. Negative control rates were 0.0005 (batch 1), 0.0007 (batch 2), 0.0006 (batch 3), 0.0006 (batch 4). Perchlorate reductase was added to cuvettes as the equivalent of 33 µL of CKB 8/3/2012 extract. ND denotes not determined, NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure B-104). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept
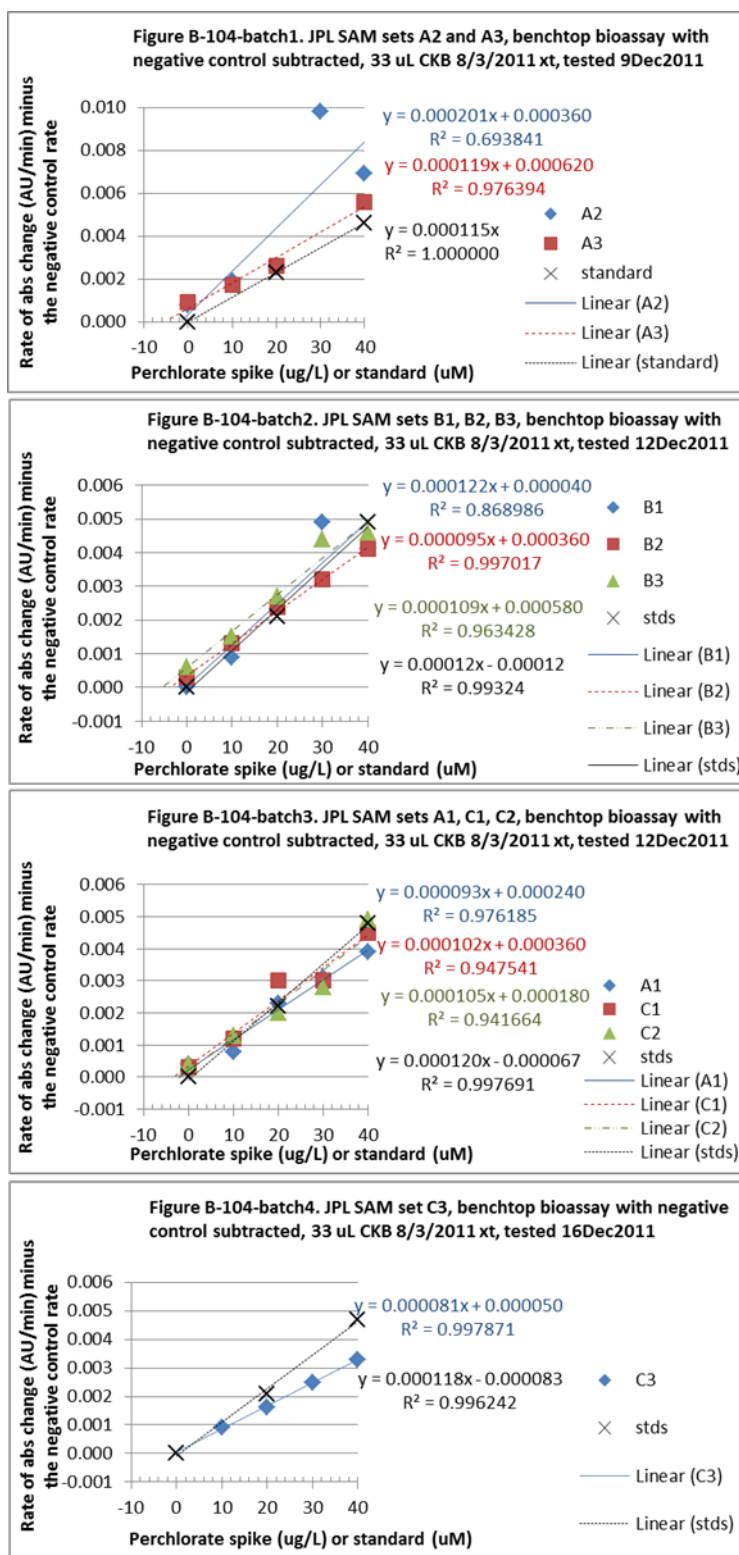
Figure B-104. Perchlorate benchtop bioassay analysis of standard additions method sets for Jet Propulsion Laboratory groundwater collected 14 Nov 2011 from location MW-16 (See Table B-104)

Groundwater sample 105, Fontana Water Company, location F-17C, collected 25Jan2012

Table B-105. Perchlorate benchtop bioassay analysis of standard additions method sets for Fontana Water Company groundwater collected 25 Jan 2012 from location F-17C[a]

Perchlorate Spike (µg/L)	Batch 1 (tested 16 Feb 2012)			Batch 2 (tested 18 Feb 2012)		
	A1	A2	A3	B1	B2	C1
0	0.0020	0.0011	0.0014	0.0006	0.0011	0.0011
10	0.0021	0.0024	0.0024	0.0015	0.0030	0.0033
20	0.0041	0.0038	0.0044	0.0035	0.0036	0.0046
30	0.0048	0.0051	0.0058	0.0046	0.0063	0.0060
40	0.0060	0.0064	0.0061	0.0052	0.0068	0.0086
Regression parameters for each set[b]						
y-intercept ± error	0.001610 ±0.000350	0.001050 ±0.000024	0.001410 ±0.000386	0.000620 ±0.000316	0.001220 ±0.000439	0.001180 ±0.000316
Slope ± error	0.000107 ±0.000014	0.000133 ±0.000001	0.000128 ±0.000016	0.000123 ±0.000013	0.000147 ±0.00018	0.000177 ±0.000013
r^2	0.9493	0.9998	0.9566	0.9681	0.9573	0.9843
x-intercept ± error	-15±4	-8±0	-11±3	-5±3	-8±3	-7±2
ClO_4^- in groundwater ± error (µg/L)[c]	15±4	8±0	11±3	5±3	8±3	7±2

Perchlorate Spike (µg/L)	Batch 3 (tested 20 Feb 2012)			Perchlorate Standards			
	B3	C2	C3	µM	Batch 1	Batch 2	Batch 3
0	0.0007	0.0010	0.0016	0	0.0000	0.0000	0.0000
10	0.0020	0.0022	0.0024	10	ND	ND	ND
20	0.0028	0.0045	0.0046	20	0.0031	0.0035	0.0039
30	0.0047	0.0069	0.0057	30	ND	ND	ND
40	0.0056	0.0056	0.0070	40	0.0061	0.0063	0.0066
Regression parameters for each set[b]							
y-intercept ± error	0.000645 ±0.000227	0.001262 ±0.000897	0.001408 ±0.000266		0.000008 ±0.000019	0.000117 ±0.000261	0.000204 ±0.000467
Slope ± error	0.000126 ±0.000009	0.000139 ±0.000037	0.000142 ±0.000011		0.000151 ±0.0000008	0.000158 ±0.000010	0.000166 ±0.000018
r^2	0.9839	0.8273	0.9828		1.0000	0.9959	0.9882
x-intercept ± error	-5±2	-9±7	-10±2		-0±0	-1±2	-1±3
ClO_4^- in groundwater ± error (µg/L)[c]	5±2	9±7	10±2		NA	NA	NA

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.00054 (batch 1), 0.0004 (batch 2), 0.000455 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 12/10/2007 extract. ND denotes not determined, NA denotes not applicable
[b]Regression parameters are from the regression line for each set (see Figure B-105). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard
[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept
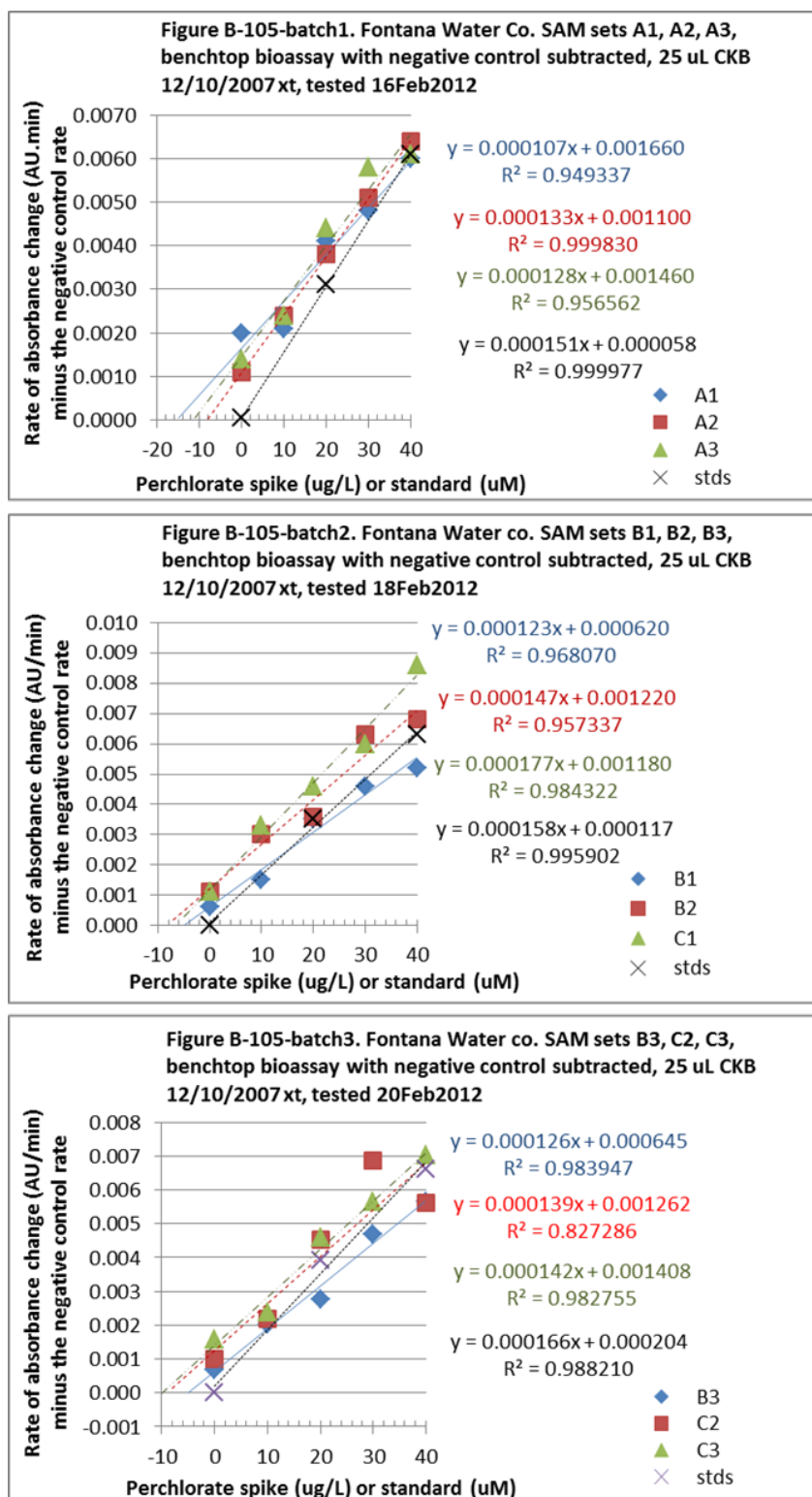
Figure B-105. Perchlorate benchtop bioassay analysis of standard additions method sets for Fontana Water Company groundwater collected 25 Jan 2012 from location F-17C (See Table B-105)

Groundwater sample 106, Massachusetts Military Reservation, location J3-INF, collected 23Feb2012

Table B-106. Perchlorate benchtop bioassay analysis of standard additions method sets for Massachusetts Military Reservation ground water collected 23 Feb 2012 from location J3-INF[a]

Perchlorate Spike (µg/L)	Batch 1 (tested 15 Mar 2012)			Batch 2 (tested 18 Mar 2012)		
	A1	**A2**	**A3**	**B1**	**B2**	**C1**
0	-0.0008	0.0032	0.0039	0.0068	0.0031	0.0136
10	0.0010	0.0061	0.0093	0.0125	0.0137	0.0154
20	0.0059	0.0074	0.0101	0.0139	0.0153	0.0172
30	0.0049	0.0082	0.0112	0.0156	0.0158	0.0186
40	0.0089	0.0105	0.0090	0.0171	0.0171	0.0203
Regression parameters for each set[b]						
y-intercept ± error	-0.000701 ±0.001127	0.003754 ±0.000469	0.006235 ±0.001833	0.008440 ±0.001161	0.006980 ±0.002751	0.013700 ±0.000102
Slope ± error	0.000233 ±0.000046	0.000166 ±0.000019	0.000123 ±0.000075	0.000237 ±0.000047	0.000301 ±0.000112	0.000166 ±0.000004
r^2	0.8951	0.9617	0.4727	0.8929	0.7054	0.9981
x-intercept ± error	3±5	-23±4	-51±34	-36±9	-23±13	-83±2
ClO_4^- in groundwater ± error (µg/L)[c]	-3±5	23±4	51±34	36±9	23±13	83±2

Perchlorate Spike (µg/L)	Batch 3 (tested 19 Mar 2012)			Perchlorate Standards			
	B3	**C2**	**C3**	**µM**	**Batch 1**	**Batch 2**	**Batch 3**
0	0.0005	0.0011	0.0009	0	0.0000	0.0000	0.0000
10	0.0028	0.0052	0.0053	10	ND	ND	ND
20	0.0027	0.0090	0.0053	20	0.0023	0.0037	0.0034
30	0.0063	0.0096	0.0063	30	ND	ND	ND
40	0.0046	0.0130	0.0069	40	0.0053	0.0070	0.0069
Regression parameters for each set[b]							
y-intercept ± error	0.001040 ±0.001041	0.001940 ±0.000849	0.002340 ±0.001037		-0.000118 ±0.000264	0.000067 ±0.000149	0.000017 ±0.000037
Slope ± error	0.000117 ±0.000043	0.000282 ±0.000035	0.000130 ±0.000042		0.000132 ±0.000010	0.000175 ±0.0000058	0.000173 ±0.0000014
r^2	0.7164	0.9566	0.7588		0.9940	0.9989	0.9999
x-intercept ± error	-9±9	-7±3	-18±10		1±2	-0±1	0±0
ClO_4^- in groundwater ± error (µg/L)[c]	9±9	7±3	18±10		NA	NA	NA

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control. Negative control rates were 0.000969 (batch 1), 0.0003 (batch 2), 0.0007 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 12/10/2007 extract. ND denotes not determined, NA denotes not applicable
[b]Regression parameters are from the regression line for each set (see Figure B-106). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard
[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept
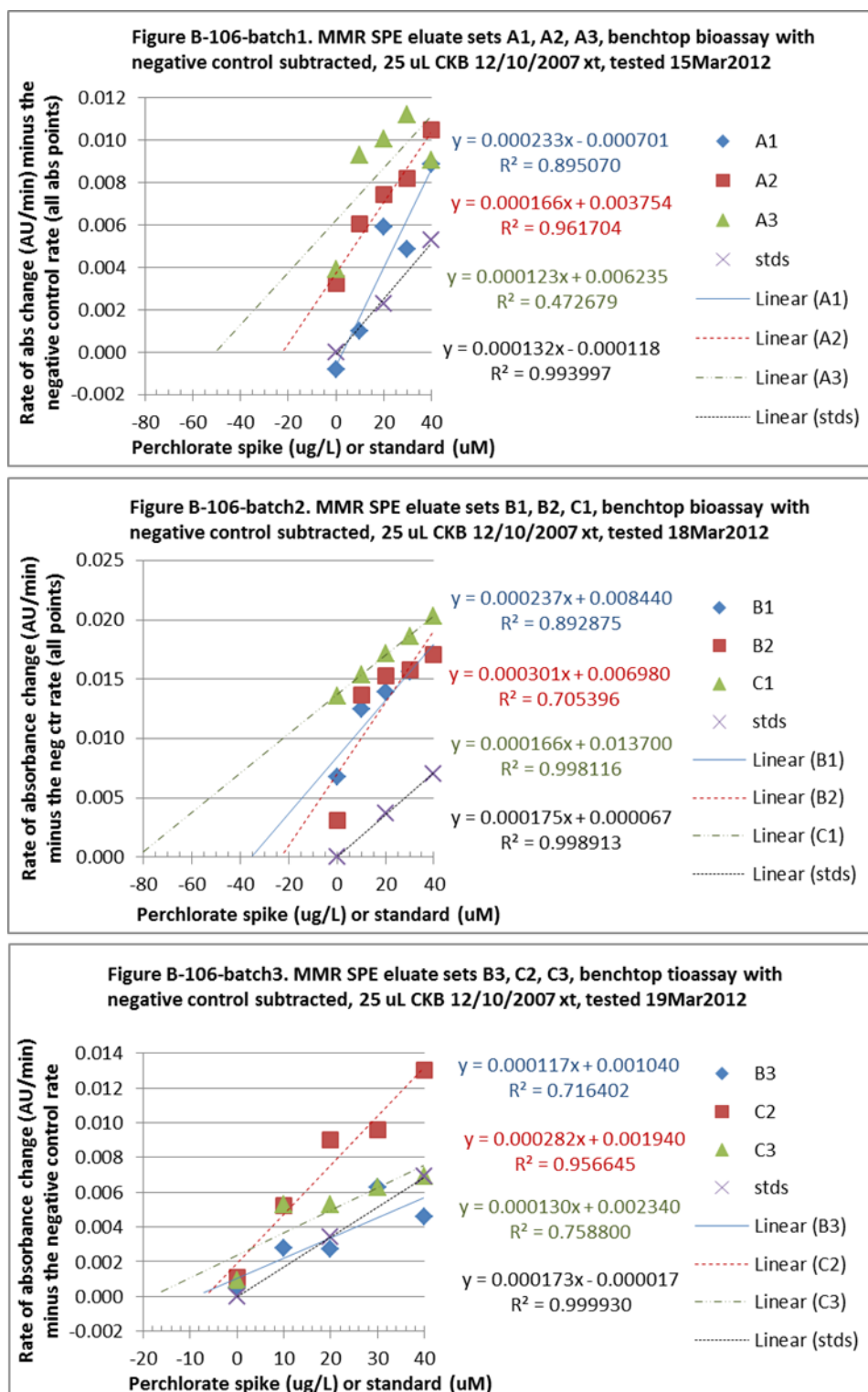
Figure B-106. Perchlorate benchtop bioassay analysis of standard additions method sets for Massachusetts Military Reservation groundwater collected 23 Feb 2012 from location J3-INF (See Table B-106)

Groundwater sample 107, Lawrence Livermore National Laboratory-site 300, location W-854-1823, collected 29 March 2012

Table B-107. Perchlorate benchtop bioassay analysis of standard additions method sets for Lawrence Livermore National Laboratory groundwater collected 29 March 2012 from location W-854-1823[a]

Perchlorate Spike (µg/L)	Batch 1 (tested 20 Apr 2012)			Batch 2 (tested 23 Apr 2012)		
	B1	**B2**	**C1**	**B3**	**C2**	**C3**
0	0.0005	0.0003	0.0002	-0.00004	0.0001	0.0003
10	0.0012	0.0011	0.0013	0.0010	0.0012	0.0017
20	0.0025	0.0016	0.0026	0.0027	0.0021	0.0029
30	0.0033	0.0030	0.0033	0.0028	0.0031	0.0035
40	0.0038	0.0041	0.0050	0.0047	0.0042	0.0056
Regression parameters for each set[b]						
y-intercept ± error	0.000520 ±0.000181	0.000120 ±0.000220	0.000154 ±0.000185	0.000048 ±0.000354	0.000106 ±0.000050	0.000284 ±0.000288
Slope ± error	0.000087 ±0.000007	0.000095 ±0.000009	0.000116 ±0.000008	0.000114 ±0.000014	0.000102 ±0.000002	0.000125 ±0.000012
r^2	0.9789	0.9738	0.9875	0.9535	0.9988	0.9739
x-intercept ± error	-6±2	-1±2	-1±2	0±3	-1±0	-2±2
ClO_4^- in groundwater ± error (µg/L)[c]	6±2	1±2	1±2	0±3	1±0	2±2

Perchlorate Spike (µg/L)	Batch 3 (tested 24 Apr 2012)			Perchlorate Standards			
	A1	**A2**	**A3**	**µM**	**Batch 1**	**Batch 2**	**Batch 3**
0	0.0002	-0.0004	-0.0003	0	0.0000	0.0000	0.0000
10	0.0014	0.0008	0.0006	10	ND	ND	ND
20	0.0024	0.0019	0.0016	20	0.0039	0.0040	0.0041
30	0.0035	0.0035	0.0029	30	ND	ND	ND
40	0.0048	0.0038	0.0036	40	0.0070	0.0068	0.0076
Regression parameters for each set[b]							
y-intercept ± error	0.000230 ±0.000054	-0.000304 ±0.000270	-0.000360 ±0.000144		0.000133 ±0.000298	0.000207 ±0.000462	0.000107 ±0.000239
Slope ± error	0.000112 ±0.000002	0.000111 ±0.000011	0.000102 ±0.000006		0.000175 ±0.000012	0.000169 ±0.000018	0.000189 ±0.0000092
r^2	0.9988	0.9714	0.9902		0.9957	0.9889	0.9976
x-intercept ± error	-2±0	3±2	4±1		-1±2	-1±3	-1±1
ClO_4^- in groundwater ± error (µg/L)[c]	2±0	-3±2	-4±1		NA	NA	NA

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.0007 (batch 1), 0.00089 (batch 2), 0.00061 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 12/10/2007 extract

[b]Regression parameters are from the regression line for each set (see Figure B-107). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept
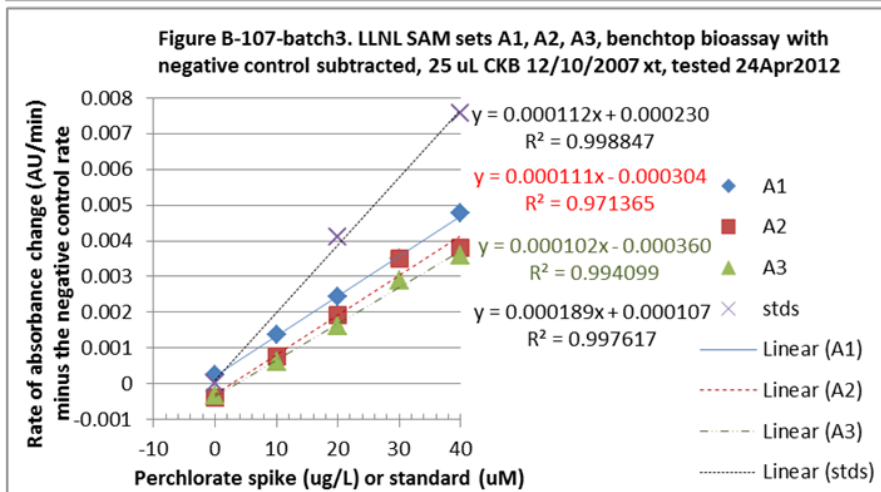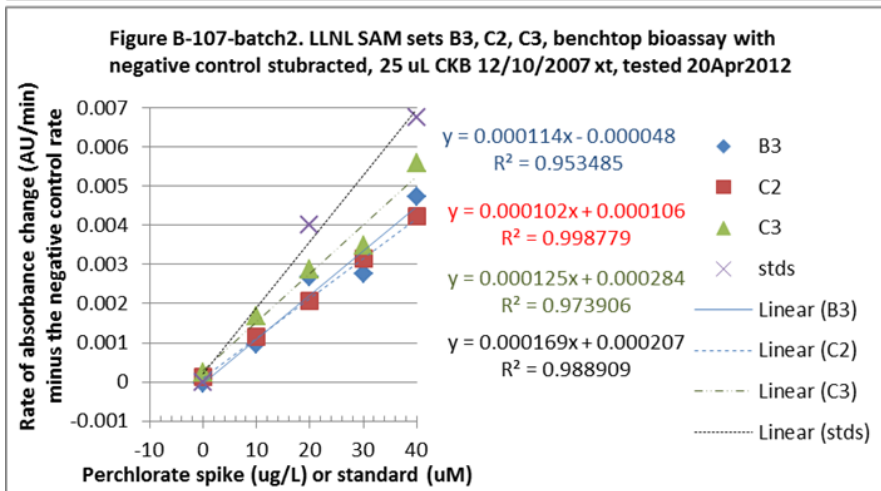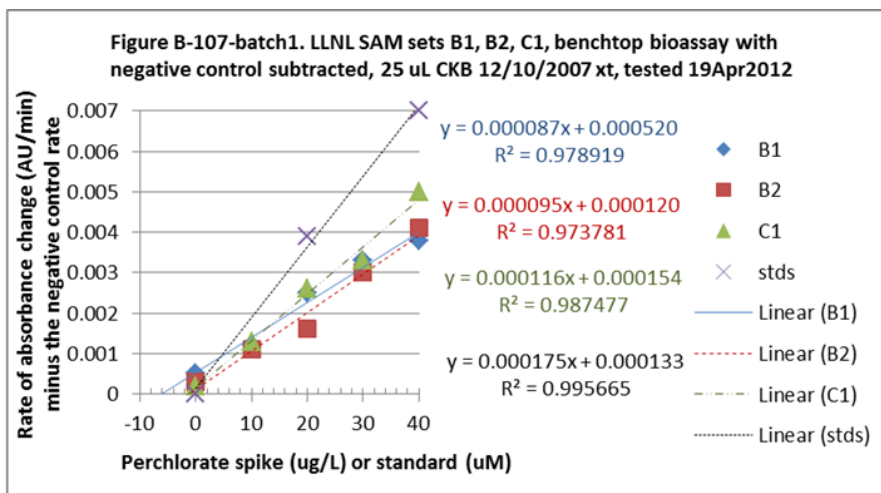
Figure B-107. Perchlorate benchtop bioassay analysis of standard additions method sets for Lawrence Livermore National Laboratory groundwater collected 29 March 2012 from location W-854-1823 (See Table 107)

Groundwater sample 102, Hill AFB, location U9-16-007, collected 14Jul2011
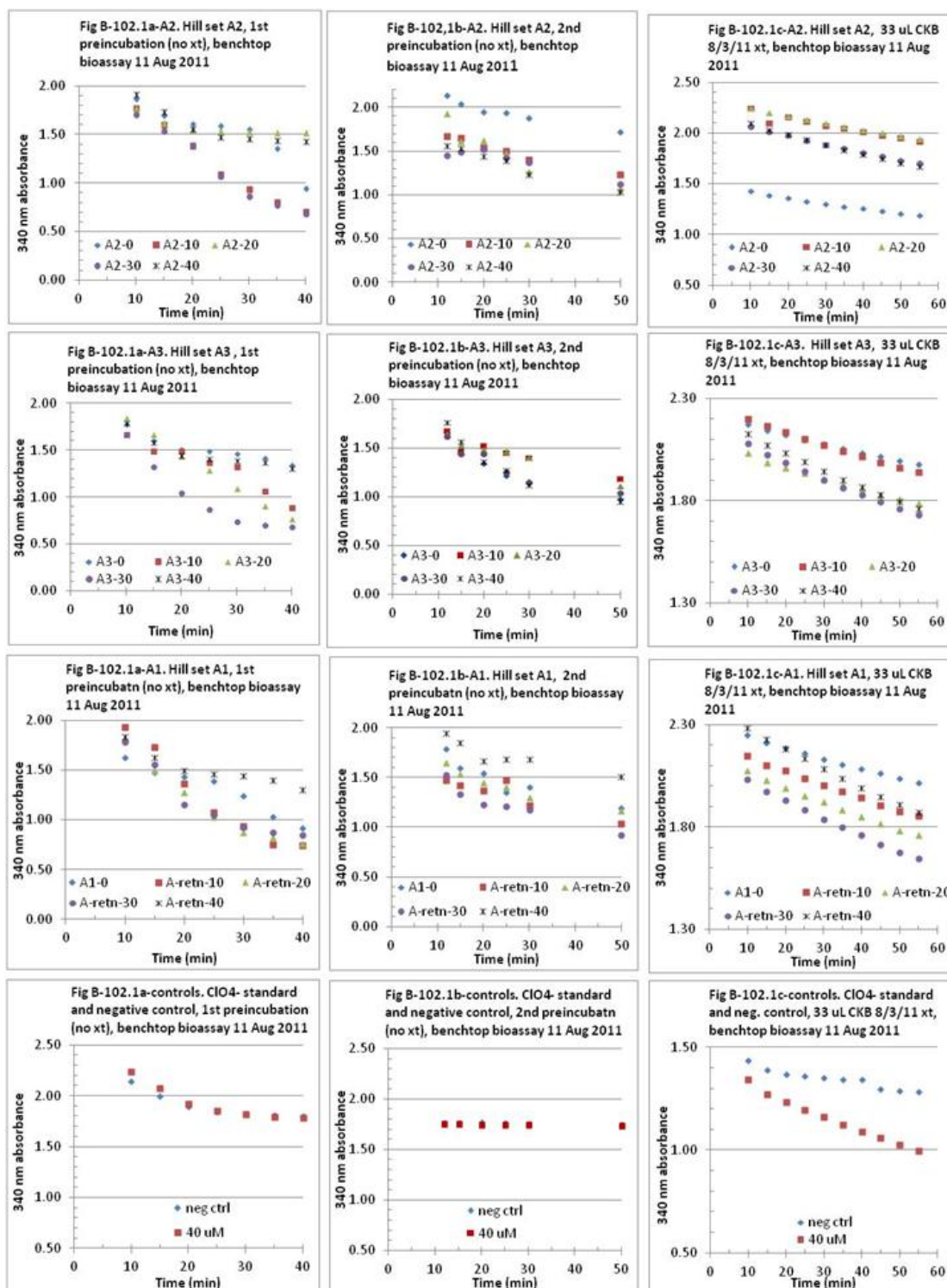
Figure B-102.1. Hill AFB, benchtop bioassay batch 1, sets A2, A3, A1, and controls. Test date 11 Aug 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-102.1a, b, and c

Data for Figure B-102.1.
Groundwater sample 102, Hill AFB. Standards addition method benchtop bioassay, batch1, 11Aug2011. SDVB/DTAB eluate sets A2, A3, A1, perchlorate 40 µM standard, and negative control (no ClO_4^- present)

Table B-102.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 11Aug2011

Time	Set A2					Set A3				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A2-0	A2-10	A2-20	A2-30	A2-40	A3-0	A3-10	A3-20	A3-30	A3-40
10	1.8625	1.7631	1.7668	1.7046	1.9052	1.7844	1.6659	1.8462	1.6736	1.7943
15	1.6924	1.5940	1.6128	1.5294	1.7266	1.6042	1.4900	1.6654	1.3228	1.5872
20	1.6023	1.3787	1.5492	1.3811	1.5488	1.5142	1.4907	1.4399	1.0436	1.4434
25	1.5835	1.0809	1.5322	1.0687	1.4733	1.4916	1.3748	1.2860	0.8689	1.4087
30	1.5503	0.9337	1.5235	0.8570	1.4472	1.4671	1.3218	1.0985	0.7374	1.3869
35	1.3525	0.7977	1.5176	0.7695	1.4339	1.4156	1.0650	0.9046	0.7063	1.3713
40	0.9420	0.7026	1.5160	0.6763	1.4254	1.3412	0.8944	0.7726	0.6811	1.3033

Time	Set A1					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctrl	40 µM
10	1.6221	1.9338	1.8169	1.7814	1.8385	2.1392	2.2380
15	1.4760	1.7303	1.4942	1.5526	1.6277	1.9991	2.0706
20	1.4332	1.3595	1.2769	1.1541	1.4912	1.8898	1.9221
25	1.3905	1.0699	1.0284	1.0468	1.4584	1.8416	1.8542
30	1.2398	0.9305	0.8696	0.9211	1.4427	1.8244	1.8175
35	1.0292	0.7479	0.8198	0.8717	1.3994	1.8108	1.7920
40	0.9167	0.7397	0.7544	0.8478	1.3032	1.7986	1.7790

Table B-102.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 11Aug2011

Time	Set A2					Set A3				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A2-0	A2-10	A2-20	A2-30	A2-40	A3-0	A3-10	A3-20	A3-30	A3-40
12	2.1339	1.6730	1.9280	1.4466	1.5559	1.6627	1.6734	1.6528	1.6206	1.7645
15	2.0353	1.6518	1.6136	1.4894	1.5295	1.5046	1.4617	1.5283	1.4465	1.5663
20	1.9470	1.5365	1.6233	1.5203	1.4364	1.3453	1.5261	1.4835	1.4428	1.3585
25	1.9402	1.5030	1.4706	1.4190	1.3935	1.2226	1.4519	1.4702	1.2573	1.2720
30	1.8736	1.3979	1.2651	1.3690	1.2297	1.1559	1.3946	1.4057	1.1463	1.1207
50	1.7186	1.2347	1.0620	1.1164	1.0318	0.9744	1.1861	1.1139	1.0423	0.9525

Time	Set A1					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctrl	40 µM
12	1.7892	1.4723	1.6463	1.5260	1.9479	1.7666	1.7510
15	1.5937	1.4220	1.5311	1.3355	1.8479	1.7651	1.7502
20	1.5400	1.3726	1.4464	1.2286	1.6648	1.7700	1.7480
25	1.3519	1.4742	1.4046	1.2125	1.6877	1.7593	1.7479
30	1.4028	1.2173	1.2963	1.1732	1.6840	1.7553	1.7466
50	1.1924	1.0354	1.1676	0.9251	1.5090	1.7470	1.7352

Table B-102.1c. Batch 1, 340 nm absorbance, 33 uL CKB 8/3/2011 extract, 11Aug2011

Time	Set A2					Set A3				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A2-0	A2-10	A2-20	A2-30	A2-40	A3-0	A3-10	A3-20	A3-30	A3-40
10	1.4337	2.2431	2.2505	2.0699	2.0968	2.1733	2.1996	2.0337	2.0792	2.1247
15	1.3862	2.0995	2.2079	2.0180	2.0342	2.1429	2.1638	1.9868	2.0254	2.0725
20	1.3607	2.1617	2.1728	1.9769	1.9886	2.1203	2.1363	1.9616	1.9844	2.0343
25	1.3317	2.1202	2.1389	1.9321	1.9350	2.0961	2.1016	1.9349	1.9430	1.9895
30	1.3002	2.0779	2.1022	1.8898	1.8855	2.0741	2.0714	1.9060	1.9009	1.9438
35	1.2771	2.0482	2.0660	1.8480	1.8358	2.0529	2.0413	1.8771	1.8607	1.9020
40	1.2565	2.0111	2.0365	1.8094	1.7931	2.0331	2.0138	1.8564	1.8281	1.8652
45	1.2338	1.9839	2.0059	1.7742	1.7512	2.0132	1.9874	1.8295	1.7947	1.8265
50	1.2115	1.9517	1.9747	1.7356	1.7094	1.9924	1.9618	1.8064	1.7589	1.7917
55	1.1928	1.9208	1.9489	1.7062	1.6732	1.9765	1.9374	1.7842	1.7284	1.7587
Rate[a]	0.0052	0.0063	0.0067	0.0081	0.0094	0.0043	0.0058	0.0054	0.0077	0.0081
r[2]	0.9869	0.9165	0.9970	0.9967	0.9961	0.9958	0.9973	0.9931	0.9950	0.9970
Rate – neg ctrl rate	0.0022	0.0033	0.0037	0.0051	0.0064	0.0013	0.0028	0.0024	0.0047	0.0051

Time	Set A1					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctrl	40 µM
10	2.2504	2.1482	2.0753	2.0344	2.2852	1.4374	1.3419
15	2.2109	2.1024	2.0270	1.9744	2.2265	1.3892	1.2730
20	2.1877	2.0731	1.9877	1.9290	2.1795	1.3699	1.2343
25	2.1608	2.0354	1.9516	1.8835	2.1325	1.3617	1.1959
30	2.1324	2.0014	1.9199	1.8381	2.0829	1.3514	1.1589
35	2.1058	1.9720	1.8825	1.7964	2.0383	1.3445	1.1241
40	2.0816	1.9412	1.8498	1.7583	1.9909	1.3411	1.0911
45	2.0613	1.9051	1.8168	1.7140	1.9473	1.2951	1.0601
50	2.0361	1.8761	1.7826	1.6762	1.9078	1.2886	1.0252
55	2.0135	1.8519	1.7585	1.6461	1.8723	1.2854	0.9972
Rate[a]	0.0052	0.0065	0.0070	0.0086	0.0092	0.0030	0.0073
r[2]	0.9959	0.9971	0.9962	0.9957	0.9974	0.9282	0.9892
Rate – neg ctrl rate	0.0022	0.0035	0.0040	0.0056	0.0062	0.0000	0.0043

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-102.1c-A1, A2, A3, and controls. r[2] is the correlation coefficient for the corresponding regression line for each reaction mixture
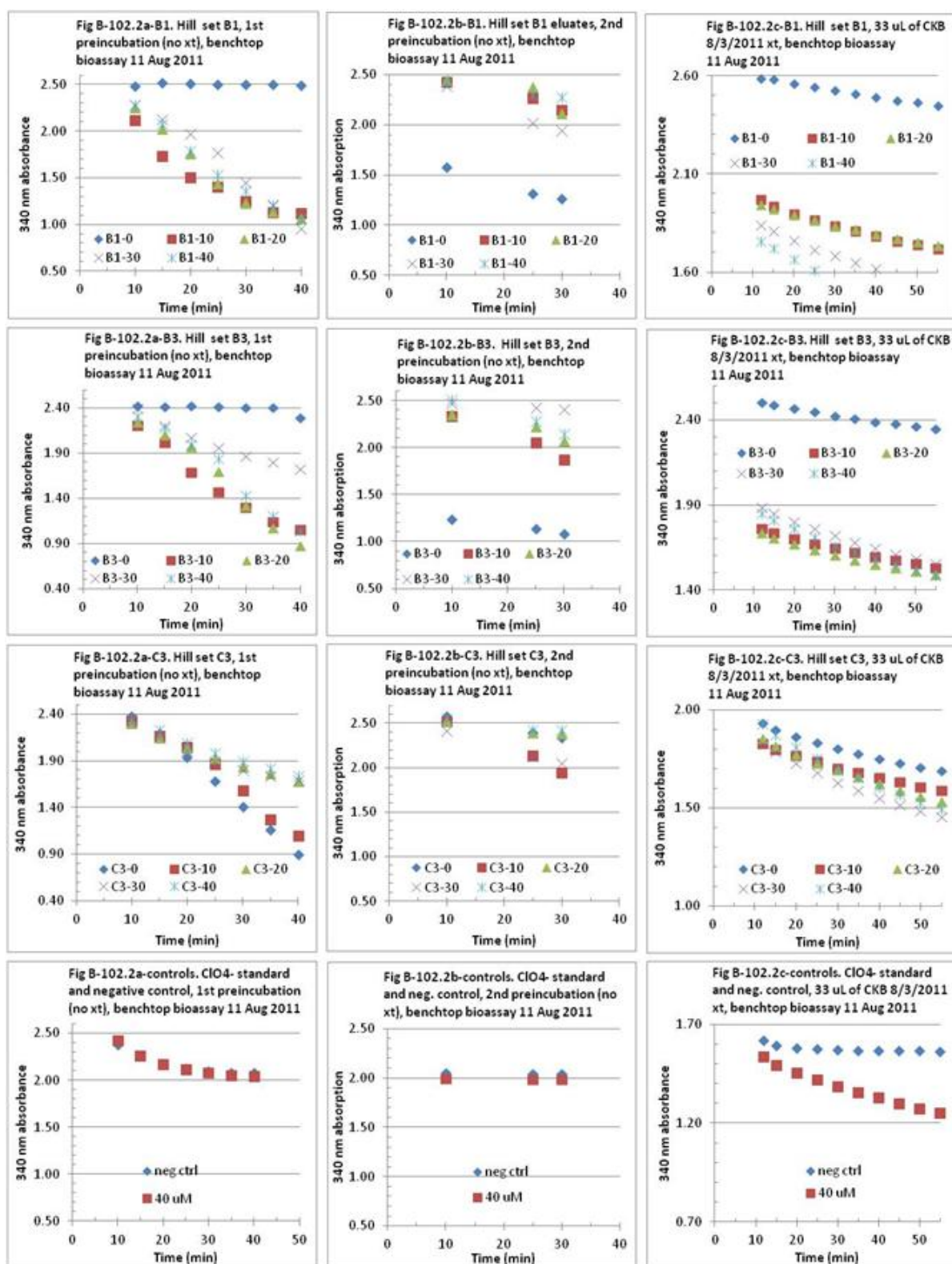
Figure B-102.2. Hill AFB, benchtop bioassay batch2, sets B1, B3, C3, and controls. Test date 11 Aug 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 μL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-102.2a, b, and c

Data for Figure B-102.2.
Groundwater sample 102, Hill AFB. Standards addition method benchtop bioassay batch2, 11Aug2011. SDVB/DTAB eluate sets B1, B3, C3, perchlorate 40 µM standard, and negative control (no ClO$_4^-$ present)

Table B-102.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 11Aug2011

Time	Set B1					Set B3				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B3-0	B3-10	B3-20	B3-30	B3-40
10	2.4859	2.1126	2.2573	2.2794	2.2803	2.4300	2.2079	2.2464	2.3950	2.3164
15	2.5237	1.7340	2.0269	2.1272	2.0842	2.4134	2.0227	2.0998	2.2091	2.1806
20	2.5059	1.5079	1.7602	1.9676	1.7891	2.4234	1.6930	1.9700	2.0773	1.9976
25	2.5025	1.4078	1.4392	1.7696	1.5353	2.4125	1.4702	1.7026	1.9597	1.8365
30	2.4997	1.2524	1.2341	1.4476	1.3558	2.4045	1.3026	1.3177	1.8693	1.4354
35	2.4968	1.1314	1.1404	1.2163	1.2009	2.4029	1.1442	1.0730	1.8060	1.2031
40	2.4931	1.1214	1.0700	0.9608	1.0667	2.2946	1.0587	0.8755	1.7247	1.0336

Time	Set C3					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctrl	40 µM
10	2.3736	2.3256	2.3038	2.3124	2.3493	2.3734	2.4259
15	2.1921	2.1675	2.1464	2.1547	2.2285	2.2560	2.2608
20	1.9307	2.0401	2.0373	2.0096	2.0934	2.1675	2.1671
25	1.6772	1.8660	1.9252	1.8915	1.9800	2.1212	2.1102
30	1.4061	1.5782	1.8350	1.7932	1.8855	2.0934	2.0757
35	1.1623	1.2644	1.7590	1.7341	1.8132	2.0797	2.0531
40	0.8922	1.0931	1.6799	1.6861	1.7372	2.0750	2.0434

TableB- 102.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 11Aug2011

Time	Set B1					Set B3				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B3-0	B3-10	B3-20	B3-30	B3-40
10	1.5777	2.4230	2.4398	2.3806	2.4226	1.2302	2.3364	2.3508	2.4721	2.5059
25	1.3095	2.2667	2.3720	2.0184	2.2992	1.1371	2.0478	2.2218	2.4245	2.2803
30	1.2563	2.1430	2.1141	1.9409	2.2686	1.0833	1.8696	2.0733	2.4046	2.1444

Time	Set C3					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctrl	40 µM
10	2.5723	2.5140	2.5154	2.4157	2.5582	2.0529	1.9909
25	2.3888	2.1379	2.3917	2.1235	2.4241	2.0487	1.9886
30	2.3352	1.9404	2.3794	2.0498	2.4192	2.0476	1.9865

Table B-102.2c. Batch 2, 340 nm absorbance, 33 uL CKB 8/3/2011 extract, 11Aug2011

Time	Set B1					Set B3				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B3-0	B3-10	B3-20	B3-30	B3-40
12	2.5867	1.9682	1.9443	1.8385	1.7582	2.5025	1.7615	1.7358	1.8853	1.8502
15	2.5825	1.9339	1.9204	1.8074	1.7212	2.4873	1.7366	1.7065	1.8489	1.8105
20	2.5619	1.8967	1.8928	1.7601	1.6642	2.4689	1.7023	1.6686	1.8033	1.7586
25	2.5423	1.8659	1.8636	1.7154	1.6103	2.4470	1.6723	1.6349	1.7616	1.7094
30	2.5252	1.8337	1.8373	1.6816	1.5612	2.4244	1.6432	1.6036	1.7186	1.6608
35	2.5080	1.8083	1.8167	1.6508	1.5223	2.4101	1.6193	1.5773	1.6818	1.6254
40	2.4899	1.7829	1.7900	1.6180	1.4799	2.3863	1.5966	1.5520	1.6433	1.5840
45	2.4753	1.7578	1.7716	1.5905	1.4432	2.3797	1.5749	1.5304	1.6123	1.5480
50	2.4644	1.7415	1.7547	1.5671	1.4114	2.3642	1.5532	1.5108	1.5841	1.5146
55	2.4489	1.7164	1.7346	1.5409	1.3771	2.3472	1.5319	1.4893	1.5544	1.4837
Rate[a]	0.0033	0.0057	0.0048	0.0068	0.0088	0.0036	0.0052	0.0056	0.0076	0.0084
r^2	0.9954	0.9839	0.9901	0.9847	0.9889	0.9901	0.9895	0.9834	0.9912	0.9903
Rate – neg ctrl rate	0.0023	0.0047	0.0038	0.0058	0.0078	0.0026	0.0042	0.0046	0.0066	0.0074

Time	Set C3					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctrl	40 µM
12	1.9315	1.8312	1.8554	1.8294	1.9283	1.6174	1.5345
15	1.9002	1.8005	1.8177	1.7851	1.8740	1.5911	1.4909
20	1.8635	1.7686	1.7718	1.7285	1.8124	1.5786	1.4522
25	1.8316	1.7365	1.7312	1.6802	1.7548	1.5734	1.4180
30	1.8010	1.7039	1.6926	1.6311	1.7000	1.5694	1.3842
35	1.7776	1.6812	1.6612	1.5910	1.6550	1.5660	1.3561
40	1.7516	1.6549	1.6263	1.5510	1.6082	1.5648	1.3270
45	1.7299	1.6324	1.5910	1.5182	1.5666	1.5649	1.2991
50	1.7077	1.6093	1.5619	1.4858	1.5306	1.5647	1.2719
55	1.6913	1.5885	1.5336	1.4554	1.4934	1.5607	1.2515
Rate[a]	0.0055	0.0055	0.0073	0.0086	0.0099	0.0010	0.0064
r^2	0.9831	0.9891	0.9917	0.9857	0.9872	0.6744	0.9854
Rate – neg ctrl rate	0.0045	0.0045	0.0063	0.0076	0.0089	0.0000	0.0054

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-102.2c-B1, B3, C3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
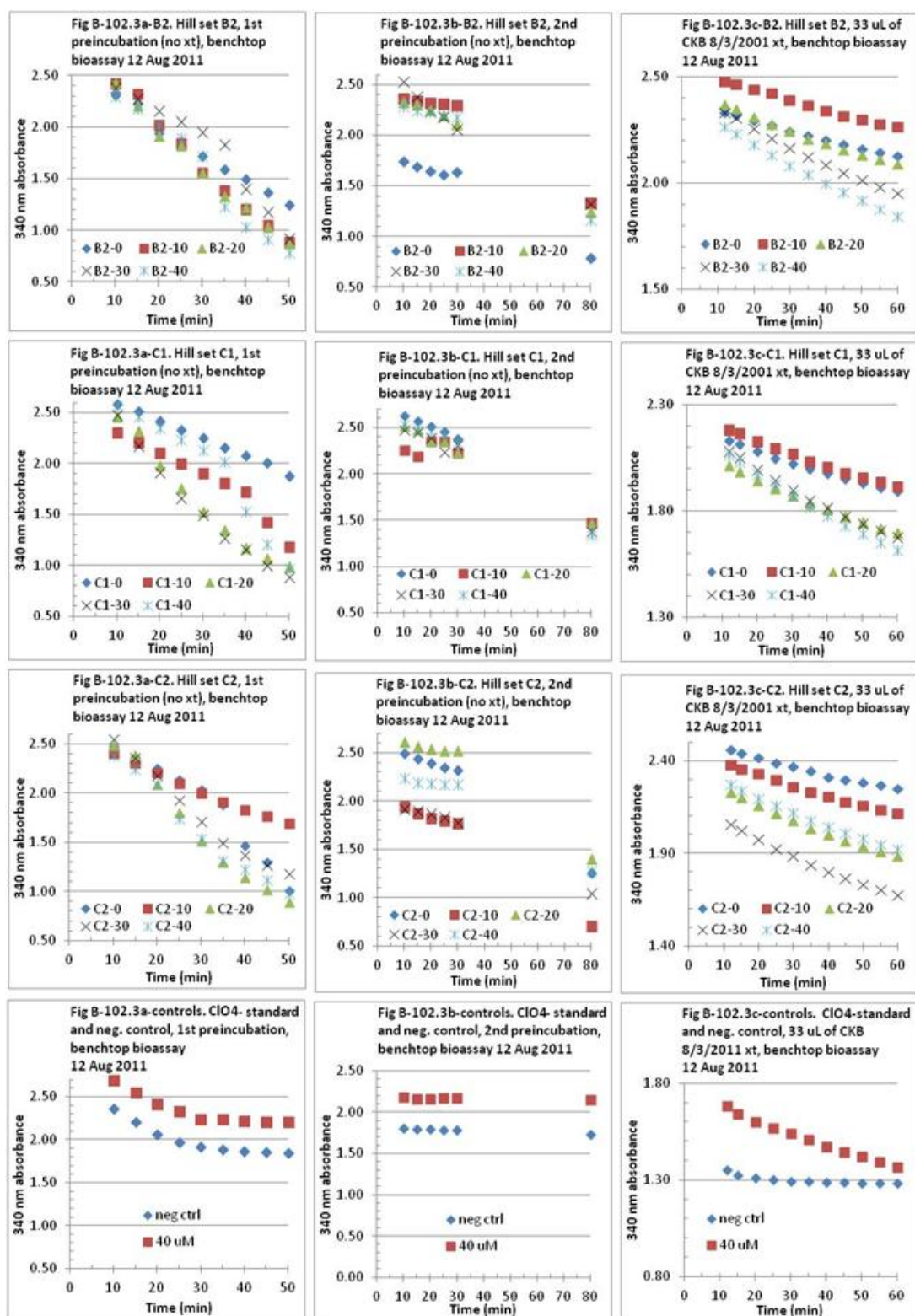
Figure B-102.3. Hill AFB, benchtop bioassay batch 3, sets B2, C1, C2, and controls. Test date 12 Aug 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-102.3a, b, and c

Data for Figure B-102.3.
Groundwater sample 102, Hill AFB. Standards addition method benchtop bioassay, batch3, 12Aug2011. SDVB/DTAB eluate sets B2, C1, C2, perchlorate 40 µM standard, and negative control (no ClO_4^- present)

Table B-102.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 12Aug2011

Time	Set B2					Set C1				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B2-0	B2-10	B2-20	B2-30	B2-40	C1-0	C1-10	C1-20	C1-30	C1-40
10	2.3134	2.4195	2.4315	2.3745	2.2926	2.5830	2.3077	2.4654	2.4781	2.5657
15	2.2200	2.3203	2.2031	2.2719	2.1715	2.5116	2.2104	2.3192	2.1787	2.4654
20	1.9419	2.0148	1.9122	2.1578	2.0208	2.4209	2.1030	1.9852	1.9181	2.3513
25	1.8475	1.8413	1.8205	2.0484	1.8863	2.3318	2.0005	1.7538	1.6662	2.2354
30	1.7188	1.5511	1.5567	1.9470	1.7177	2.2513	1.9058	1.5292	1.4963	2.1296
35	1.5870	1.3814	1.3315	1.8326	1.2278	2.1585	1.8125	1.3472	1.2719	2.0158
40	1.4931	1.2006	1.2190	1.3968	1.0311	2.0818	1.7200	1.1761	1.1568	1.5353
45	1.3689	1.0474	1.0301	1.1730	0.9118	2.0086	1.4307	1.0662	1.0032	1.2058
50	1.2490	0.8870	0.8727	0.9208	0.7853	1.8799	1.1834	0.9928	0.8889	0.9633

Time	Set C2					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	C2-0	C2-10	C2-20	C2-30	C2-40	neg ctrl	40 µM
10	2.4780	2.4038	2.4852	2.5482	2.3803	2.3652	2.6926
15	2.3744	2.3048	2.3890	2.3610	2.2458	2.2023	2.5497
20	2.2519	2.2037	2.0922	2.1801	2.0945	2.0636	2.4157
25	2.1390	2.0966	1.8028	1.9269	1.7369	1.9709	2.3288
30	2.0360	2.0006	1.5143	1.7142	1.5442	1.9168	2.2407
35	1.8882	1.9068	1.2964	1.5002	1.3187	1.8824	2.2400
40	1.4716	1.8275	1.1413	1.3689	1.2287	1.8634	2.2153
45	1.3011	1.7682	1.0228	1.2755	1.1139	1.8536	2.2112
50	1.0065	1.6930	0.8927	1.1771	0.9807	1.8450	2.2047

Table B-102.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 12Aug2011

Time	Set B2					Set C1				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B2-0	B2-10	B2-20	B2-30	B2-40	C1-0	C1-10	C1-20	C1-30	C1-40
10	1.7453	2.3666	2.3337	2.5291	2.2828	2.6303	2.2625	2.5150	2.4757	2.5335
15	1.6905	2.3409	2.3012	2.3846	2.2458	2.5692	2.1945	2.4789	2.4534	2.4869
20	1.6430	2.3236	2.2485	2.2334	2.2306	2.5158	2.3529	2.3529	2.3913	2.4633
25	1.6143	2.3144	2.1954	2.1816	2.1990	2.4545	2.3578	2.3578	2.2385	2.4209
30	1.6363	2.2954	2.1122	2.0586	2.1813	2.3842	2.2313	2.2313	2.3175	2.3405
80	0.7864	1.3310	1.2459	1.3230	1.1678	1.4480	1.4756	1.4756	1.3737	1.3415

Time	Set A1					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	C2-0	C2-10	C2-20	C2-30	C2-40	neg ctrl	40 µM
10	2.4959	1.9391	2.6133	1.9124	2.2372	1.8060	2.1811
15	2.4343	1.8689	2.5638	1.8958	2.1935	1.8020	2.1664
20	2.3951	1.8237	2.5363	1.8664	2.1843	1.7956	2.1628
25	2.3486	1.7944	2.5245	1.8286	2.1703	1.7902	2.1747
30	2.3227	1.7661	2.5167	1.7751	2.1729	1.7847	2.1692
80	1.2512	0.7018	1.4017	1.0449	1.2719	1.7357	2.1469

TableB- 102.3c. Batch 3, 340 nm absorbance, 33 uL CKB 8/3/2011 extract, 12Aug2011

Time	Set B2					Set C1				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B2-0	B2-10	B2-20	B2-30	B2-40	C1-0	C1-10	C1-20	C1-30	C1-40
10	2.3334	2.4789	2.3716	2.3344	2.2674	2.1316	2.1794	2.0125	2.0811	2.0648
15	2.3194	2.4662	2.3500	2.3055	2.2326	2.1126	2.1633	1.9854	2.0506	2.0294
20	2.2932	2.4409	2.3099	2.2557	2.1827	2.0776	2.1264	1.9428	1.9926	1.9726
25	2.2741	2.4222	2.2768	2.2098	2.1334	2.0482	2.0917	1.9047	1.9424	1.9201
30	2.2442	2.3913	2.2468	2.1675	2.0841	2.0225	2.0651	1.8696	1.8960	1.8697
35	2.2232	2.3665	2.2069	2.1242	2.0390	1.9959	2.0306	1.8336	1.8479	1.8188
40	2.2047	2.3405	2.1845	2.0861	1.9986	1.9752	2.0047	1.8044	1.8119	1.7749
45	2.1818	2.3161	2.1583	2.0490	1.9554	1.9501	1.9769	1.7739	1.7724	1.7296
50	2.1630	2.2979	2.1325	2.0165	1.9175	1.9311	1.9552	1.7455	1.7388	1.6899
55	2.1435	2.2782	2.1120	1.9831	1.8779	1.9094	1.9326	1.7175	1.7035	1.6494
60	2.1296	2.2640	2.0898	1.9548	1.8440	1.8933	1.9141	1.6946	1.6732	1.6131
Rate[a]	0.0043	0.0047	0.0059	0.0080	0.0088	0.0050	0.0056	0.0066	0.0085	0.0094
r^2	0.9956	0.9954	0.9899	0.9936	0.9961	0.9908	0.9922	0.9925	0.9907	0.9953
Rate – neg ctrl rate	0.0032	0.0036	0.0048	0.0069	0.0077	0.0039	0.0045	0.0055	0.0074	0.0083

Time	Set C2					Controls	
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17
(min)	C2-0	C2-10	C2-20	C2-30	C2-40	neg ctrl	40 µM
10	2.4598	2.3776	2.2327	2.0568	2.2742	1.3522	1.6854
15	2.4432	2.3568	2.2031	2.0240	2.2393	1.3252	1.6436
20	2.4166	2.3305	2.1601	1.9763	2.1962	1.3112	1.6012
25	2.3869	2.2998	2.1169	1.9264	2.1577	1.3031	1.5701
30	2.3711	2.2585	2.0768	1.8847	2.1211	1.2970	1.5418
35	2.3440	2.2308	2.0343	1.8394	2.0777	1.2931	1.5089
40	2.3130	2.2064	2.0002	1.8021	2.0462	1.2908	1.4739
45	2.2988	2.1768	1.9685	1.7649	2.0101	1.2881	1.4450
50	2.2852	2.1595	1.9400	1.7347	1.9810	1.2869	1.4229
55	2.2675	2.1364	1.9106	1.7033	1.9479	1.2867	1.3937
60	2.2511	2.1182	1.8845	1.6742	1.9225	1.2864	1.3701
Rate[a]	0.0044	0.0055	0.0073	0.0080	0.0073	0.0011	0.0063
r^2	0.9881	0.9906	0.9914	0.9911	0.9941	0.7214	0.9891
Rate – neg ctrl rate	0.0033	0.0044	0.0062	0.0069	0.0062	0.0000	0.0052

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-102.3c-B2, C1, C2, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture

Groundwater sample 103, NWIRP McGregor, location OFFWS-37, collected 13Oct2011
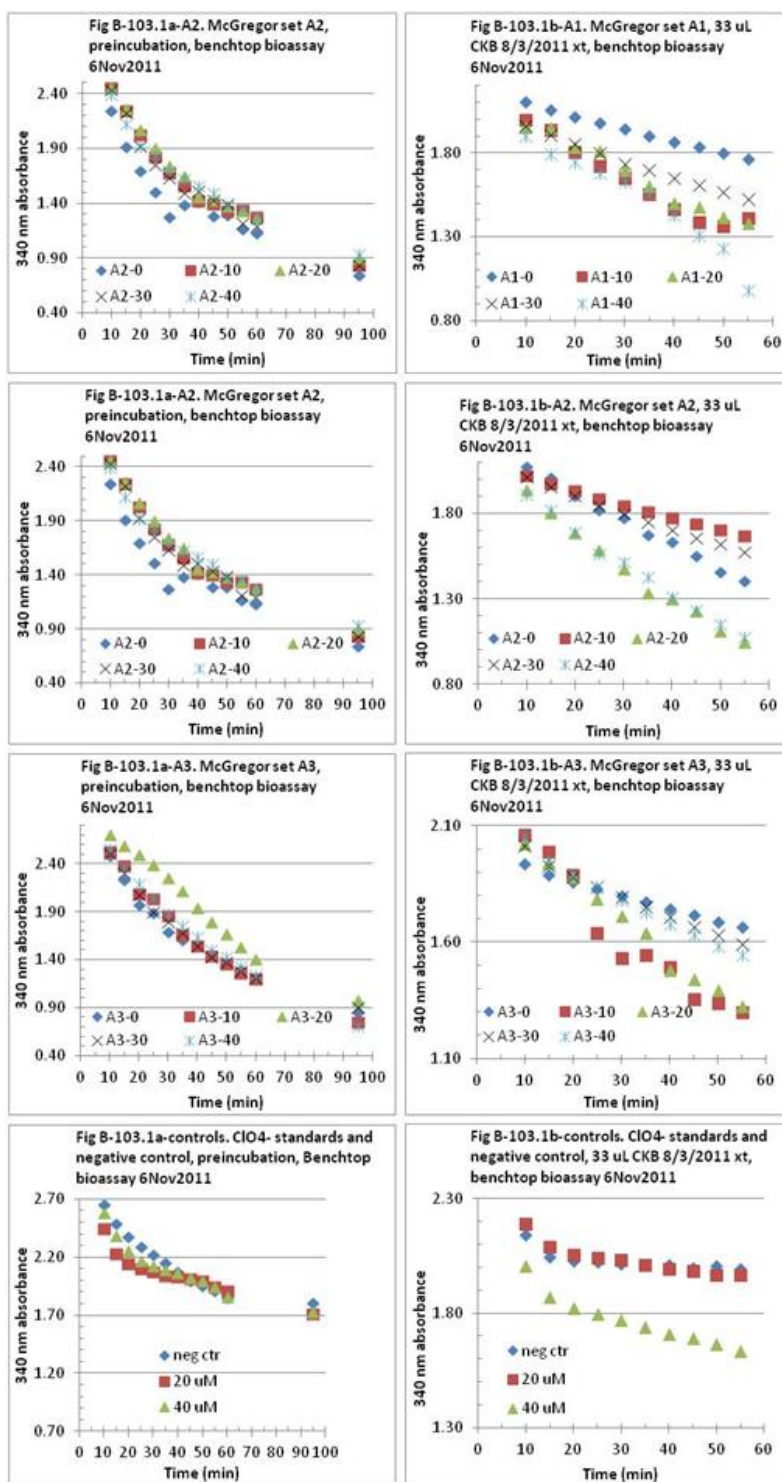
Figure B-103.1. NWIRP McGregor, benchtop bioassay batch 1, sets A1, A2, A3, and controls. Test date 6 Nov 2011. Preincubation without extract (**a**), 33 μL CKB 8/3/2011 extract (**b**). Data values displayed are listed in Tables B-103.1a and b

Data for Figure B-103.1.
Groundwater sample 103, NWIRP McGregor. Standards addition method benchtop bioassay, batch1, 6Nov2011. SDVB/DTAB eluate sets A1, A2, A3, perchlorate 20 and 40 µM standards, and negative control (no ClO_4^- present)

Table B-103.1a. Batch 1, 340 nm absorbance, preincubation without extract, 6Nov2011

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.2645	2.3078	2.2392	2.3064	2.4394	2.2466	2.4591	2.4589	2.4332	2.396
15	2.0144	2.0811	2.0734	2.0437	2.2297	1.9145	2.2483	2.2671	2.2269	2.1274
20	1.8236	1.8082	1.9306	1.7740	2.0732	1.6968	2.0284	2.0677	1.9298	1.9210
25	1.6299	1.6840	1.7853	1.7711	1.8700	1.5096	1.8247	1.9060	1.7508	1.8312
30	1.6129	1.6055	1.7528	1.6203	1.7678	1.2757	1.6760	1.7423	1.6366	1.7011
35	1.5801	1.4855	1.6115	1.5698	1.6993	1.3855	1.5629	1.6497	1.4967	1.6224
40	1.4800	1.4026	1.6044	1.5218	1.5407	1.4135	1.4186	1.4609	1.5147	1.5634
45	1.3185	1.3807	1.4984	1.4296	1.6040	1.2854	1.3967	1.4295	1.4338	1.5001
50	1.3149	1.3839	1.4726	1.3002	1.5211	1.2923	1.3342	1.3788	1.3929	1.3758
55	1.2469	1.1947	1.3731	1.2686	1.4269	1.1712	1.3362	1.3328	1.2175	1.3635
60	1.2434	1.2435	1.2916	1.2373	1.4122	1.1280	1.2723	1.2699	1.1957	1.2673
95	0.9023	0.9566	0.9756	0.9665	0.9882	0.7441	0.8351	0.9015	0.8380	0.9386

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 µM	40 µM
10	2.4897	2.5215	2.7024	2.5045	2.5565	2.6513	2.4469	2.5813
15	2.2345	2.3818	2.5927	2.3117	2.3649	2.4855	2.2320	2.3887
20	1.9780	2.0834	2.4973	2.0762	2.1973	2.3790	2.1466	2.2565
25	1.8933	2.0390	2.3864	1.8995	2.0480	2.2949	2.0974	2.1665
30	1.6888	1.8546	2.2571	1.7959	1.8839	2.2239	2.0755	2.1277
35	1.6056	1.6545	2.1202	1.6804	1.7537	2.1488	2.0438	2.0900
40	1.5489	1.5398	1.9397	1.5401	1.6373	2.0722	2.0348	2.0666
45	1.4458	1.4361	1.7987	1.4289	1.4979	1.9948	2.0148	2.0242
50	1.3661	1.3541	1.6734	1.3729	1.4258	1.9564	2.0003	1.9993
55	1.2991	1.2584	1.5380	1.2864	1.3277	1.9116	1.9441	1.9419
60	1.2122	1.2018	1.4138	1.2065	1.2414	1.8502	1.9117	1.8616
95	0.8522	0.7494	0.9774	0.9062	0.7069	1.8055	1.7164	1.7307

Table B-103.1b. Batch 1, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 6Nov2011

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.1022	1.9965	1.9541	1.9588	1.9009	2.0717	2.0194	1.9409	2.0157	1.9109
15	2.0540	1.9320	1.9437	1.9018	1.7903	2.0072	1.9717	1.8045	1.9573	1.8235
20	2.0134	1.8028	1.8333	1.8486	1.7426	1.9064	1.9334	1.6853	1.9035	1.6928
25	1.9760	1.7164	1.8069	1.7995	1.6791	1.8201	1.8864	1.5869	1.8474	1.5616
30	1.9415	1.6499	1.6924	1.7298	1.6237	1.7761	1.8479	1.4742	1.7975	1.5141
35	1.9005	1.5544	1.6002	1.6969	1.5607	1.6778	1.8086	1.3342	1.7505	1.4263
40	1.8642	1.4604	1.4997	1.6498	1.4245	1.6311	1.7725	1.2997	1.7042	1.3118
45	1.8306	1.3858	1.4748	1.6052	1.3044	1.5523	1.7374	1.2323	1.6555	1.2336
50	1.7975	1.3593	1.4170	1.5611	1.2296	1.4571	1.7035	1.1147	1.6202	1.1508
55	1.7624	1.4097	1.3775	1.5211	0.9788	1.4065	1.6709	1.0460	1.5742	1.0752
Rate[a]	0.0074	0.0148	0.0142	0.0097	0.0185	0.0149	0.0077	0.0196	0.0098	0.0186
r^2	0.9980	0.9475	0.9799	0.9955	0.9537	0.9963	0.9969	0.9875	0.9966	0.9935
Rate – neg ctrl rate	0.0052	0.0126	0.0120	0.0075	0.0163	0.0127	0.0055	0.0174	0.0076	0.0164

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 μM	40 μM
10	1.9371	2.0630	2.0212	2.0138	2.0541	2.1422	2.1893	2.0068
15	1.8877	1.9871	1.9427	1.9272	1.9476	2.0482	2.0894	1.8723
20	1.8573	1.8910	1.8820	1.8806	1.8909	2.0271	2.0567	1.8221
25	1.8277	1.6396	1.7873	1.8363	1.8369	2.0221	2.0428	1.7969
30	1.7983	1.5316	1.7116	1.7937	1.7831	2.0166	2.0314	1.7692
35	1.7708	1.5462	1.6402	1.7522	1.7316	2.0123	2.0127	1.7405
40	1.7409	1.4951	1.4803	1.7070	1.6799	2.0109	1.9924	1.7099
45	1.7169	1.3540	1.4435	1.6662	1.6316	1.9999	1.9827	1.6895
50	1.6856	1.3371	1.3930	1.6291	1.5837	2.0080	1.9681	1.6633
55	1.6655	1.2994	1.3246	1.5907	1.5434	1.9948	1.9673	1.6359
Rate[a]	0.0059	0.0176	0.0161	0.0090	0.0109	0.0022	0.0041	0.0070
r^2	0.9936	0.9308	0.9897	0.9898	0.9883	0.5851	0.8399	0.9095
Rate – neg ctrl rate	0.0037	0.0154	0.0139	0.0068	0.0087	0.0000	0.0019	0.0048

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-103.1b-A1, A2, A3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
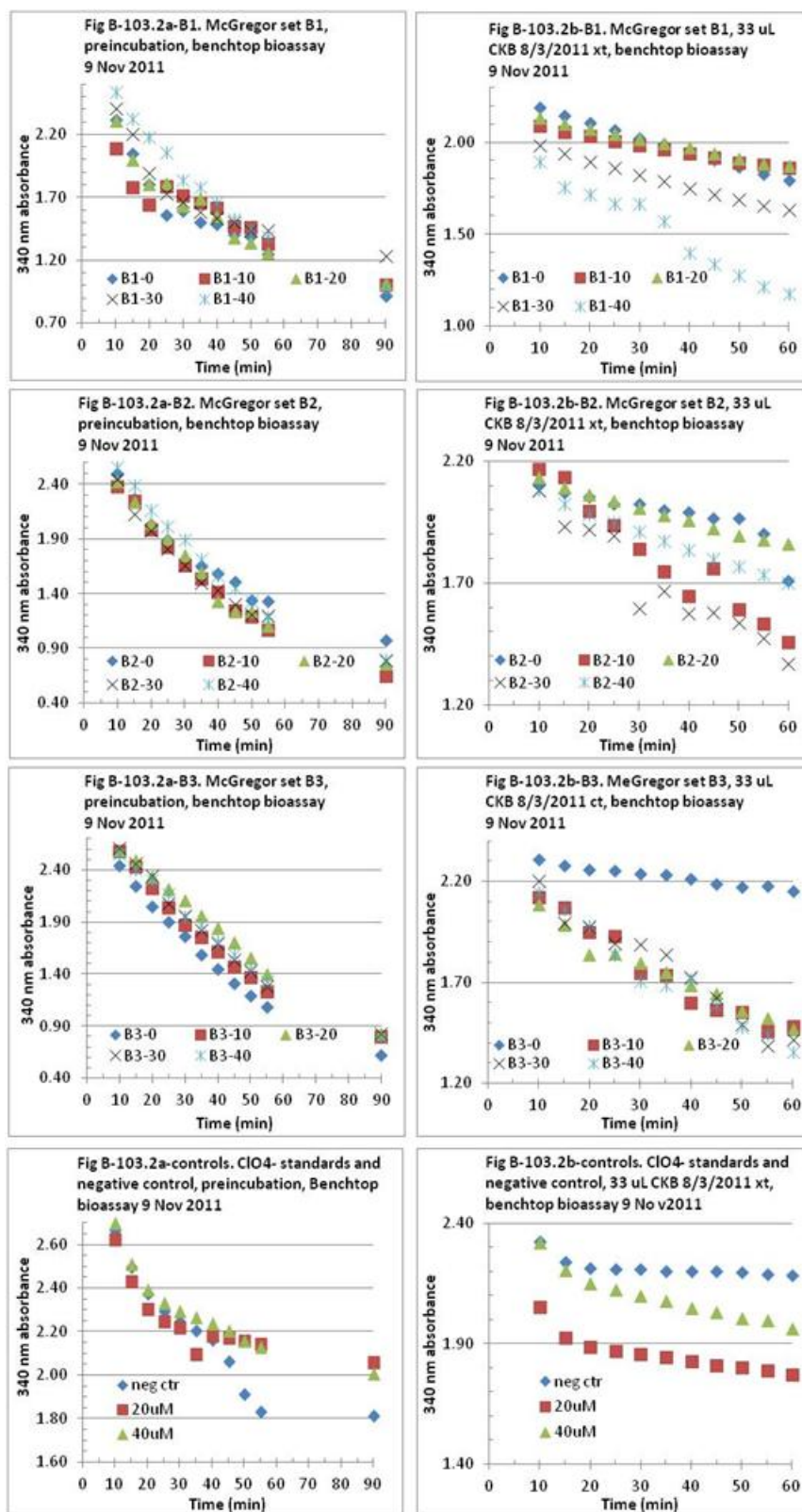
Figure B-103.2. NWIRP McGregor, benchtop bioassay batch 2, sets B1, B2, B3, and perchlorate controls. Test date 9 Nov 2011. Preincubation without extract (**a**), 33 µL CKB 8/3/2011 extract (**b**). Data values displayed are listed in Tables B-103.2 a and b.

Data for Figure B-103.2.
Groundwater sample 103, NWIRP McGregor. Standards addition method benchtop bioassay, batch 2, 9Nov2011. SDVB/DTAB eluate sets B1, B2, B3, perchlorate 20 and 40 µM standards, and negative control (no ClO4- present)

Table B-103.2a. Batch 2, 340 nm absorbance, preincubation without extract, 9Nov2011

	Set B1					Set B2				
Time	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.3139	2.0903	2.3112	2.4031	2.5373	2.4912	2.3778	2.4279	2.4463	2.5585
15	2.0500	1.7817	2.0029	2.2015	2.3227	2.2670	2.2445	2.2385	2.1265	2.3891
20	1.8034	1.6416	1.8049	1.8930	2.1787	2.0275	1.9859	2.0375	1.9760	2.1636
25	1.5596	1.7873	1.8119	1.7323	2.0544	1.8897	1.8278	1.9041	1.8095	2.0094
30	1.5959	1.7117	1.6299	1.6684	1.8352	1.7159	1.6580	1.7511	1.6601	1.8897
35	1.5028	1.6607	1.6879	1.5840	1.7807	1.6436	1.5371	1.5917	1.5013	1.7147
40	1.4826	1.6197	1.5515	1.5357	1.6566	1.5808	1.4089	1.3282	1.4283	1.5618
45	1.4072	1.4727	1.3841	1.5052	1.5288	1.5043	1.2464	1.2382	1.3056	1.4507
50	1.3862	1.4588	1.3395	1.4370	1.4487	1.3416	1.1912	1.2315	1.2070	1.3304
55	1.2513	1.3308	1.2561	1.4353	1.3906	1.3309	1.0668	1.1048	1.1937	1.1796
90	0.9158	1.0072	1.0116	1.2340	0.9761	0.9721	0.6452	0.7547	0.7878	0.7928

	Set B3					Controls		
Time	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	neg ctr	20 µM	40 µM
10	2.4353	2.5778	2.5881	2.6034	2.5456	2.6659	2.6265	2.6973
15	2.2468	2.4314	2.4854	2.4584	2.4011	2.4957	2.4331	2.5110
20	2.0428	2.2203	2.3327	2.3448	2.3046	2.3742	2.3040	2.3916
25	1.9015	2.0390	2.2108	2.0781	2.1601	2.2954	2.2474	2.3315
30	1.7594	1.8703	2.1096	1.9592	1.9520	2.2471	2.2164	2.2926
35	1.5850	1.7531	1.9588	1.8175	1.8661	2.2050	2.0964	2.2668
40	1.4503	1.6139	1.8385	1.6872	1.7109	2.1621	2.1790	2.2397
45	1.3086	1.4699	1.6969	1.5382	1.5829	2.0639	2.1727	2.2030
50	1.1894	1.3703	1.5554	1.4228	1.4450	1.9119	2.1561	2.1579
55	1.0808	1.2275	1.3969	1.2834	1.3264	1.8309	2.1429	2.1293
90	0.6181	0.7947	0.8297	0.8273	0.7822	1.8134	2.0603	2.0080

Table B-103.2b. Batch 2, 340 nm absorbance, 33uL CKB 8/3/2011 extract, 9Nov2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.1911	2.0876	2.1359	1.9860	1.8945	2.1021	2.1659	2.1318	2.0767	2.0811
15	2.1486	2.0582	2.1027	1.9402	1.7535	2.0711	2.1325	2.0931	1.9328	2.0233
20	2.1075	2.0359	2.0755	1.8973	1.7143	2.0518	1.9944	2.0633	1.9170	1.9825
25	2.0670	2.0076	2.0479	1.8603	1.6679	2.0225	1.9341	2.0351	1.8957	1.9477
30	2.0258	1.9849	2.0186	1.8214	1.6681	2.0218	1.8407	2.0065	1.5943	1.9085
35	1.9897	1.9631	1.9965	1.7866	1.5709	2.0003	1.7462	1.9793	1.6669	1.8712
40	1.9513	1.9407	1.9706	1.7511	1.4014	1.9914	1.6460	1.9560	1.5761	1.8351
45	1.9078	1.9190	1.9385	1.7182	1.3350	1.9666	1.7582	1.9211	1.5799	1.7977
50	1.8663	1.8910	1.9100	1.6910	1.2780	1.9655	1.5935	1.8919	1.5381	1.7658
55	1.8282	1.8780	1.8851	1.6545	1.2141	1.9014	1.5334	1.8788	1.4752	1.7332
60	1.7945	1.8592	1.8754	1.6328	1.1768	1.7089	1.4558	1.8599	1.3706	1.7026
Rate[a]	0.0080	0.0046	0.0053	0.0071	0.0145	0.0055	0.0140	0.0055	0.0130	0.0074
r^2	0.9996	0.9963	0.9962	0.9947	0.9716	0.7499	0.9574	0.9936	0.9138	0.9957
Rate – neg ctrl rate	0.0062	0.0028	0.0035	0.0053	0.0127	0.0037	0.0122	0.0037	0.0112	0.0056

Time	Set B3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	neg ctr	20 μM	40 μM
10	2.3063	2.1212	2.0860	2.2011	2.1433	2.3231	2.0520	2.3226
15	2.2765	2.0692	1.9818	1.9903	2.0568	2.2377	1.9253	2.2060
20	2.2577	1.9457	1.8358	1.9707	1.9751	2.2164	1.8849	2.1511
25	2.2504	1.9258	1.8417	1.8920	1.8348	2.2086	1.8712	2.1258
30	2.2382	1.7450	1.7936	1.8845	1.7021	2.2087	1.8573	2.1009
35	2.2286	1.7346	1.7497	1.8349	1.6838	2.2010	1.8430	2.0765
40	2.2121	1.5952	1.6849	1.7220	1.7116	2.2008	1.8286	2.0496
45	2.1846	1.5631	1.6408	1.6242	1.5847	2.1995	1.8113	2.0297
50	2.1716	1.5512	1.5544	1.4922	1.4755	2.1975	1.8022	2.0064
55	2.1750	1.4569	1.5211	1.3859	1.4384	2.1869	1.7885	1.9968
60	2.1507	1.4824	1.4663	1.4140	1.3534	2.1829	1.7710	1.9638
Rate[a]	0.0029	0.0140	0.0114	0.0154	0.0153	0.0018	0.0043	0.0060
r^2	0.9785	0.9549	0.9718	0.9607	0.9686	0.5885	0.8109	0.9078
Rate – neg ctrl rate	0.0011	0.0122	0.0096	0.0136	0.0135	0.0000	0.0025	0.0042

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-103.2b-B1, B2, B3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture

Groundwater sample 104, Jet Propulsion Laboratory (JPL), location MW-16, collected 14Nov2011
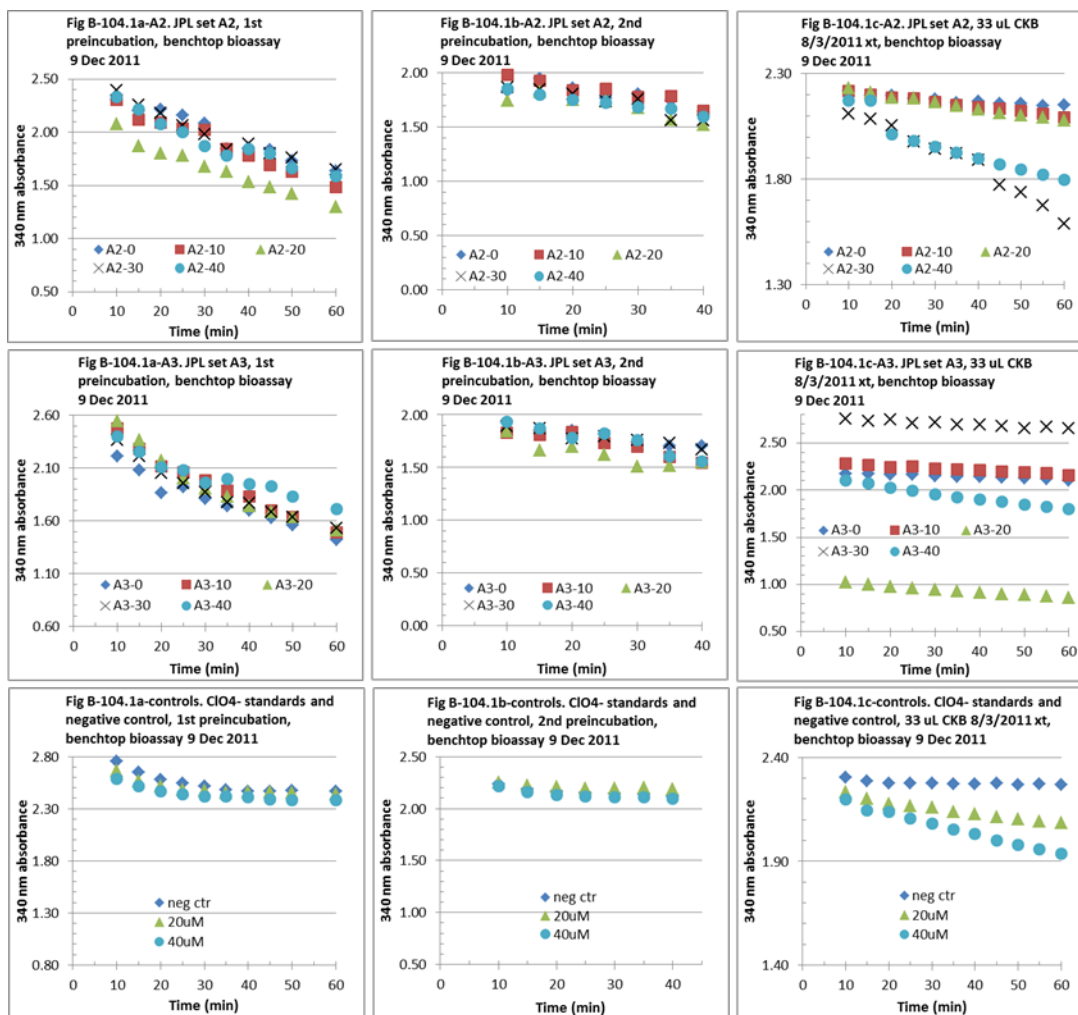
Figure B-104.1. Jet Propulsion Laboratory, benchtop bioassay batch 1, sets A1, A2, A3, and perchlorate controls. Test date 9 Dec 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 μL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.1a, b, and c

Data for Figure B-104.1.

Groundwater sample 104, Jet Propulsion Laboratory. Standards addition method benchtop bioassay, batch 1, 9Dec2011. SDVB/DTAB eluate sets A2, A3, perchlorate 20 and 40 μM standards, and negative control (no ClO_4^- present). With respect to set A1, the 340 nm absorption time course was aberrant for A1-0, A1-10 and A1-20. Testing was performed again for set A1 in batch 3 on 12Dec2011.

Table B-104.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 9Dec2011

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.3943	2.3416	2.1412	2.1757	2.1370	2.3049	2.3059	2.0737	2.3926	2.3293
15	2.1682	2.2454	2.0449	2.0763	1.9785	2.1986	2.1160	1.8683	2.2594	2.2128
20	2.1829	2.2619	2.0939	2.0772	1.9287	2.2180	2.0920	1.7979	2.1801	2.0740
25	2.1048	2.0375	1.9508	2.0233	1.9130	2.1586	2.0326	1.7805	2.0617	2.0029
30	2.0610	2.0058	1.9037	1.8546	1.7703	2.0820	2.0236	1.6740	1.9800	1.8692
35	1.8877	1.9809	1.8353	1.7701	1.6535	1.8399	1.8421	1.6252	1.8382	1.7831
40	1.7825	1.8259	1.7920	1.7253	1.5459	1.8483	1.7822	1.5317	1.8882	1.8426
45	1.8005	1.7662	1.6840	1.6857	1.4965	1.8353	1.6874	1.4845	1.7983	1.8039
50	1.6480	1.6895	1.4631	1.6353	1.4392	1.7337	1.6257	1.4211	1.7623	1.6645
60	1.6000	1.6436	1.5053	1.4924	1.3313	1.6363	1.4815	1.2987	1.6525	1.5884

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 μM	40 μM
10	2.2121	2.4792	2.5494	2.3661	2.4031	2.7556	2.6647	2.5831
15	2.0789	2.2814	2.3642	2.2173	2.2570	2.6463	2.5620	2.5126
20	1.8641	2.1190	2.1694	2.0560	2.1129	2.5797	2.5135	2.4683
25	1.9229	2.0587	2.0028	1.9574	2.0833	2.5460	2.4812	2.4410
30	1.8126	1.9844	1.9077	1.8760	1.9605	2.5175	2.4710	2.4199
35	1.7406	1.8863	1.8291	1.7763	2.0010	2.4792	2.4523	2.4177
40	1.6988	1.8346	1.7411	1.7670	1.9494	2.4674	2.4597	2.4107
45	1.6291	1.6979	1.6836	1.6834	1.9266	2.4686	2.4575	2.3921
50	1.5618	1.6334	1.6370	1.6358	1.8282	2.4706	2.4505	2.3851
60	1.4214	1.4922	1.5139	1.5307	1.7113	2.4638	2.4407	2.3809

Table B-104.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 9Dec2011

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	1.8524	1.8397	1.7184	1.7051	1.8537	1.8323	1.9801	1.7471	1.8704	1.8546
15	1.8350	1.8002	1.7106	1.7685	1.7968	1.9487	1.9253	1.8276	1.8442	1.7973
20	1.8017	1.8737	1.6901	1.7166	1.7244	1.8609	1.8418	1.7552	1.8065	1.7545
25	1.7685	1.7564	1.5684	1.6259	1.7396	1.7673	1.8510	1.7361	1.7396	1.7284
30	1.7118	1.7094	1.5348	1.6629	1.7239	1.8076	1.7774	1.6747	1.7604	1.6839
35	1.6092	1.6266	1.5262	1.5125	1.6908	1.7482	1.7848	1.5705	1.5627	1.6687
40	1.6076	1.5120	1.4880	1.4199	1.6563	1.6452	1.6490	1.5239	1.5633	1.5978

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 μM	40 μM
10	1.9299	1.8286	1.8401	1.8889	1.9329	2.2387	2.2522	2.2118
15	1.8487	1.8121	1.6649	1.8715	1.8718	2.1872	2.2231	2.1560
20	1.8536	1.8352	1.6956	1.7756	1.7787	2.1675	2.2084	2.1297
25	1.8140	1.7356	1.6185	1.7928	1.8213	2.1511	2.1950	2.1188
30	1.7411	1.6966	1.5063	1.7587	1.7592	2.1429	2.1976	2.1124
35	1.7017	1.6032	1.5166	1.7299	1.6070	2.1400	2.1980	2.1110
40	1.7061	1.5441	1.5526	1.6720	1.5595	2.1188	2.1901	2.0987

Table B-104.1c. Batch 1, 340 nm absorbance, 33 µL CKB 8/3/2011 extract, 9Dec2011

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.2190	2.3045	2.0855	2.2512	2.0401	2.2168	2.2180	2.2299	2.1103	2.1725
15	2.1680	2.2950	2.0635	2.2217	2.0087	2.1980	2.2016	2.2095	2.0861	2.1729
20	2.1243	2.2656	2.0538	2.2057	1.9812	2.1969	2.1862	2.1851	2.0556	2.0125
25	2.0914	2.2102	2.0274	2.1779	1.9431	2.1838	2.1816	2.1830	1.9778	1.9801
30	2.0145	2.1592	2.0099	2.1563	1.9188	2.1810	2.1646	2.1649	1.9423	1.9543
35	1.9456	2.0426	1.9950	2.1345	1.8922	2.1617	2.1516	2.1476	1.9222	1.9239
40	1.9136	2.0280	1.9754	2.1163	1.8644	2.1675	2.1413	2.1296	1.8896	1.8966
45	1.9269	1.8435	1.9519	2.0995	1.8400	2.1595	2.1325	2.1123	1.7733	1.8693
50	1.9381	1.8067	1.8939	2.0750	1.8196	2.1602	2.1223	2.1032	1.7376	1.8456
55	1.8506	1.7941	1.8146	2.0618	1.7920	2.1476	2.1089	2.0917	1.6738	1.8205
60	1.8460	1.7159	1.7405	2.0462	1.7749	2.1509	2.0932	2.0784	1.5893	1.7978
Rate[a]	0.0075	0.0131	0.0062	0.0041	0.0053	0.0013	0.0024	0.0030	0.0103	0.0074
r^2	0.9327	0.9631	0.8887	0.9944	0.9947	0.9190	0.9950	0.9911	0.9748	0.9101
Rate – neg ctrl rate	0.0070	0.0126	0.0057	0.0036	0.0048	0.0008	0.0019	0.0025	0.0098	0.0069

	Set A3					Controls		
Time	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 µM	40 µM
10	2.1725	2.2827	1.0230	2.7528	2.1074	2.3044	2.2329	2.1989
15	2.1729	2.2640	0.9989	2.7371	2.0758	2.2871	2.2007	2.1458
20	2.1641	2.2414	0.9771	2.7452	2.0226	2.2749	2.1759	2.1363
25	2.1661	2.2494	0.9619	2.7092	1.9918	2.2772	2.1651	2.1047
30	2.1490	2.2301	0.9473	2.7164	1.9588	2.2762	2.1579	2.0807
35	2.1407	2.2230	0.9317	2.6938	1.9265	2.2734	2.1372	2.0530
40	2.1397	2.2104	0.9146	2.6948	1.8997	2.2704	2.1265	2.0319
45	2.1310	2.1959	0.9017	2.6810	1.8747	2.2747	2.1143	2.0005
50	2.1271	2.1881	0.8879	2.6587	1.8466	2.2684	2.1014	1.9791
55	2.1223	2.1827	0.8766	2.6697	1.8233	2.2712	2.0914	1.9592
60	2.1010	2.1596	0.8635	2.6570	1.8013	2.2677	2.0843	1.9378
Rate[a]	0.0014	0.0022	0.0031	absorbance	0.0061	0.0005	0.0028	0.0051
r^2	0.9536	0.9765	0.9907	out of	0.9883	0.6282	0.9691	0.9925
Rate – neg ctrl rate	0.0009	0.0017	0.0026	range for assay	0.0056	0.0000	0.0023	0.0046

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.1c-A1, A2, A3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
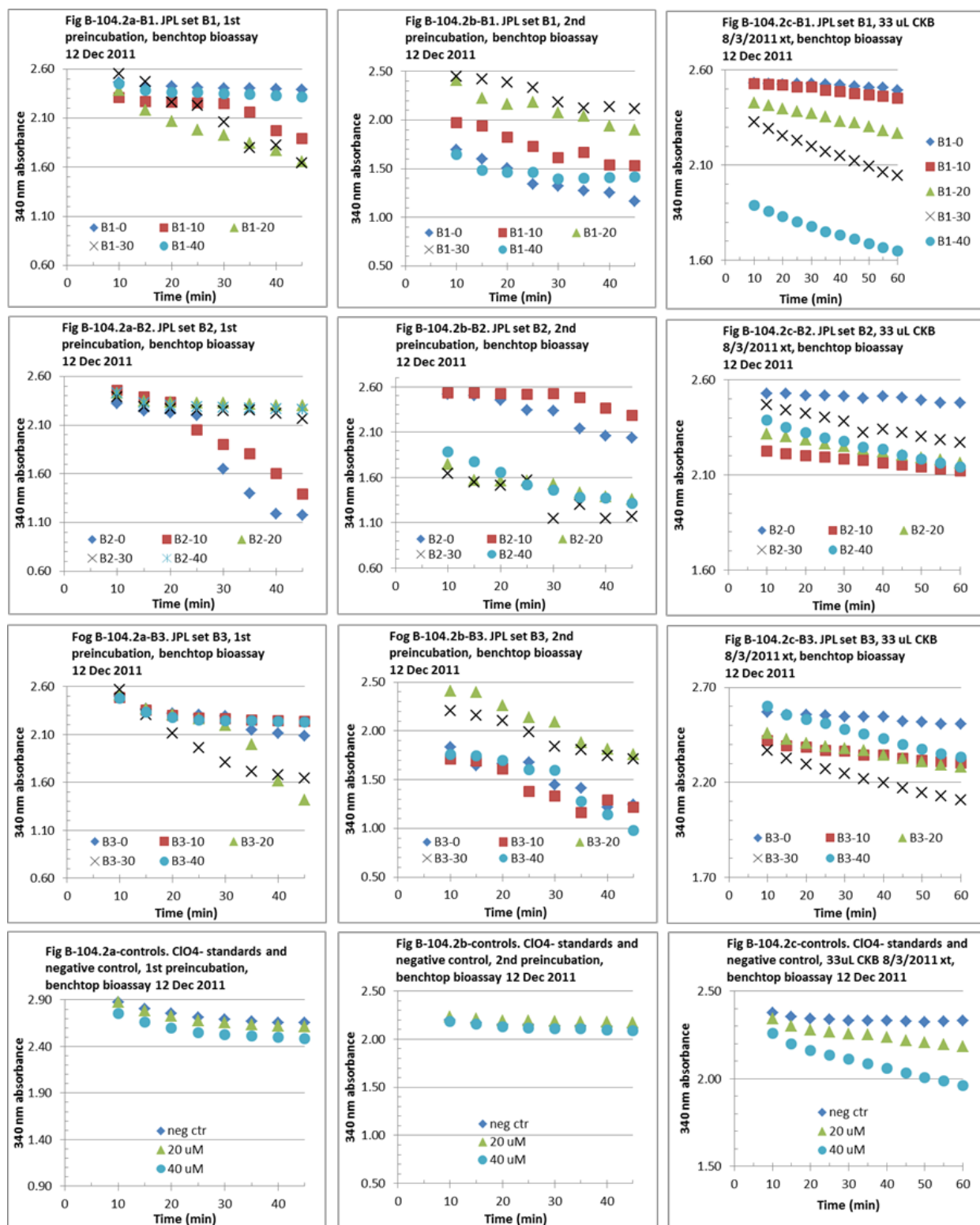
Figure B-104.2. Jet Propulsion Laboratory, benchtop bioassay batch 2, sets B1, B2, B3, and perchlorate controls. Test date 12 Dec 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 μL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.2a, b, and c

Data for Figure B-104.2.
Groundwater sample 104, JPL. Standards addition method benchtop bioassay, batch 2, 12Dec2011. SDVB/DTAB eluate sets B1, B2, B3, perchlorate 20 and 40 µM standards, and negative control (no ClO$_4^-$ present).

Table B-104.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 12Dec2011

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.4634	2.3118	2.3850	2.5522	2.4483	2.3237	2.4587	2.4320	2.3882	2.4304
15	2.4249	2.2708	2.1852	2.4715	2.3841	2.2487	2.3873	2.3517	2.2881	2.3248
20	2.4226	2.2615	2.0699	2.2627	2.3645	2.2295	2.3359	2.3360	2.2679	2.2978
25	2.4122	2.2563	1.9821	2.2301	2.3631	2.1991	2.0521	2.3290	2.2534	2.2874
30	2.4073	2.2471	1.9270	2.0610	2.3502	1.6504	1.9024	2.3250	2.2477	2.2824
35	2.4022	2.1628	1.8424	1.7954	2.3442	1.3987	1.8040	2.3160	2.2569	2.2745
40	2.3951	1.9716	1.7716	1.8280	2.3307	1.1905	1.6051	2.3032	2.2219	2.2698
45	2.3908	1.8932	1.6596	1.6419	2.3170	1.1732	1.3913	2.3024	2.1676	2.2627

Time	Set B3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	neg ctr	20 µM	40 µM
10	2.4969	2.4832	2.5119	2.5638	2.4740	2.8765	2.8713	2.7535
15	2.3660	2.3539	2.3637	2.3034	2.3281	2.8016	2.7854	2.6625
20	2.3191	2.2961	2.3020	2.1131	2.2743	2.7520	2.7285	2.5952
25	2.3006	2.2683	2.2656	1.9573	2.2509	2.7137	2.6752	2.5513
30	2.2890	2.2601	2.1916	1.8067	2.2443	2.6876	2.6582	2.5273
35	2.1479	2.2483	1.9954	1.7125	2.2395	2.6704	2.6340	2.5116
40	2.1105	2.2435	1.6178	1.6797	2.2315	2.6545	2.6178	2.4959
45	2.0799	2.2363	1.4153	1.6395	2.2265	2.6515	2.6097	2.4848

Table B-104.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 12Dec2011

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	1.6961	1.9759	2.4111	2.4506	1.6441	2.5178	2.5299	1.7467	1.6392	1.8851
15	1.6003	1.9399	2.2250	2.4196	1.4857	2.5032	2.5285	1.5702	1.5450	1.7732
20	1.5027	1.8210	2.1626	2.3893	1.4666	2.4543	2.5243	1.5641	1.5140	1.6551
25	1.3449	1.7259	2.1867	2.3325	1.4657	2.3438	2.5205	1.5645	1.5697	1.5190
30	1.3243	1.6165	2.0783	2.1805	1.3978	2.3317	2.5211	1.5236	1.1427	1.4572
35	1.2736	1.6661	2.0396	2.1239	1.4013	2.1363	2.4837	1.4307	1.3006	1.3791
40	1.2504	1.5397	1.9380	2.1348	1.4097	2.0553	2.3646	1.3881	1.1490	1.3718
45	1.1630	1.5311	1.8968	2.1146	1.4186	2.0393	2.2812	1.3592	1.1713	1.3140

Time	Set B3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	neg ctr	20 μM	40 μM
10	1.8305	1.7089	2.4114	2.2029	1.7606	2.1945	2.2302	2.1858
15	1.6461	1.6899	2.3948	2.1572	1.7426	2.1709	2.2147	2.1550
20	1.6635	1.6074	2.2593	2.1021	1.7008	2.1524	2.1940	2.1258
25	1.6793	1.3757	2.1353	1.9920	1.6035	2.1464	2.1876	2.1125
30	1.4478	1.3287	2.0895	1.8396	1.5949	2.1335	2.1873	2.1073
35	1.4154	1.1610	1.8822	1.8092	1.2775	2.1342	2.1768	2.1052
40	1.2143	1.2909	1.8105	1.7433	1.1434	2.1305	2.1743	2.0947
45	1.2427	1.2128	1.7561	1.7094	0.9808	2.1181	2.1724	2.0887

Table B-104.2c. Batch 2, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 12Dec2011

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.5335	2.5297	2.4267	2.3264	1.8881	2.5269	2.2250	2.3171	2.4682	2.3893
15	2.5298	2.5254	2.4133	2.2933	1.8565	2.5257	2.2101	2.3018	2.4390	2.3488
20	2.5255	2.5230	2.3959	2.2542	1.8294	2.5166	2.2019	2.2852	2.4225	2.3235
25	2.5287	2.5129	2.3822	2.2297	1.8022	2.5159	2.1925	2.2644	2.4019	2.2959
30	2.5270	2.5098	2.3706	2.1970	1.7758	2.5148	2.1819	2.2505	2.3815	2.2757
35	2.5240	2.4949	2.3554	2.1715	1.7512	2.5021	2.1761	2.2364	2.3240	2.2468
40	2.5214	2.4876	2.3321	2.1487	1.7308	2.5133	2.1627	2.2218	2.3410	2.2367
45	2.5136	2.4782	2.3230	2.1232	1.7098	2.5049	2.1507	2.2050	2.3219	2.2057
50	2.5087	2.4695	2.3029	2.0940	1.6883	2.4930	2.1415	2.1889	2.3024	2.1830
55	2.5078	2.4632	2.2823	2.0643	1.6667	2.4780	2.1314	2.1793	2.2826	2.1641
60	2.4945	2.4513	2.2683	2.0440	1.6476	2.4794	2.1211	2.1642	2.2720	2.1405
Rate[a]	0.0007	0.0016	0.0032	0.0056	0.0048	0.0010	0.0020	0.0031	0.0039	0.0048
r^2	0.8750	0.9848	0.9960	0.9964	0.9948	0.8739	0.9974	0.9972	0.9655	0.9931
Rate – neg ctrl rate	0.0000	0.0009	0.0025	0.0049	0.0041	0.0003	0.0013	0.0024	0.0032	0.0041

	Set B3					Controls		
Time	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	neg ctr	20 μM	40 μM
10	2.5710	2.4200	2.4588	2.3692	2.6019	2.3773	2.3433	2.2582
15	2.5578	2.3918	2.4290	2.3256	2.5551	2.3529	2.3001	2.1986
20	2.5575	2.3843	2.4083	2.2966	2.5326	2.3423	2.2785	2.1636
25	2.5517	2.3692	2.3898	2.2710	2.5112	2.3405	2.2684	2.1366
30	2.5454	2.3647	2.3784	2.2452	2.4813	2.3323	2.2577	2.1109
35	2.5462	2.3444	2.3694	2.2193	2.4539	2.3317	2.2534	2.0856
40	2.5447	2.3453	2.3444	2.1994	2.4299	2.3316	2.2364	2.0595
45	2.5232	2.3284	2.3269	2.1714	2.3982	2.3290	2.2202	2.0326
50	2.5197	2.3178	2.3096	2.1465	2.3764	2.3239	2.2080	2.0065
55	2.5071	2.3107	2.2913	2.1279	2.3523	2.3280	2.1970	1.9866
60	2.5069	2.3028	2.2819	2.1074	2.3345	2.3335	2.1848	1.9599
Rate[a]	0.0013	0.0022	0.0034	0.0051	0.0053	0.0007	0.0028	0.0056
r^2	0.9575	0.9759	0.9914	0.9927	0.9950	0.6239	0.9580	0.9857
Rate – neg ctrl rate	0.0006	0.0015	0.0027	0.0044	0.0046	0.0000	0.0021	0.0049

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.2c-B1, B2, B3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
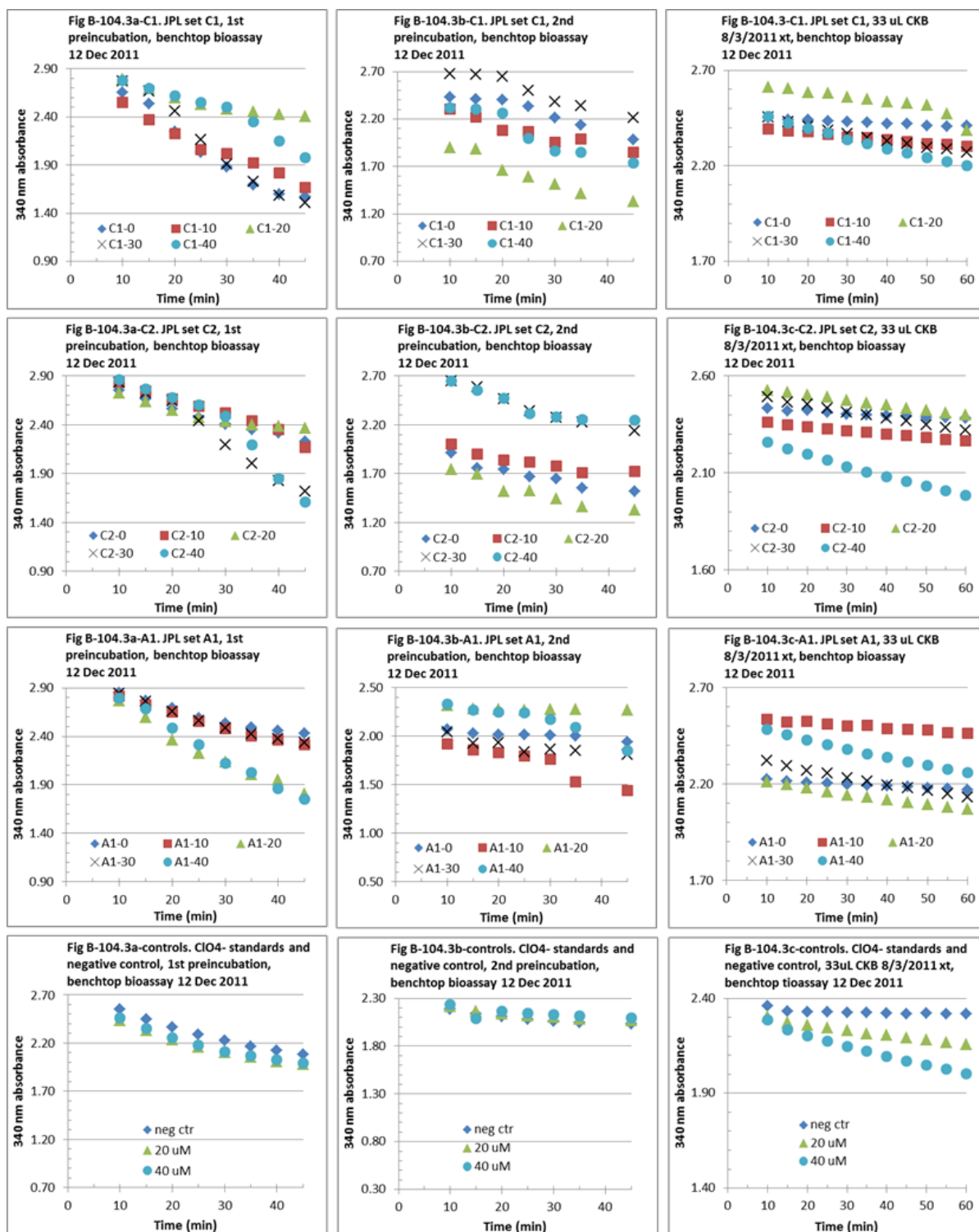
Figure B-104.3. Jet Propulsion Laboratory, benchtop bioassay batch 3, sets C1, C2, A1, and perchlorate controls. Test date 12 Dec 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 μL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.3a, b, and c

Data for Figure B-104.3.

Groundwater sample 104, JPL. Standards addition method benchtop bioassay, batch 3, 12Dec2011. SDVB/DTAB eluate sets C1, C2, A1 (repeated 9Dec2011 testing), perchlorate 20 and 40 μM standards, and negative control (no ClO_4^- present).

Table B-104.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 12Dec2011

	Set C1					Set C2				
Time	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	2.6527	2.5525	2.7903	2.7720	2.7782	2.7564	2.8326	2.7287	2.8348	2.8590
15	2.5382	2.3727	2.6994	2.6710	2.6969	2.6624	2.7464	2.6341	2.7247	2.7646
20	2.2440	2.2267	2.6019	2.4588	2.6195	2.5630	2.6653	2.5465	2.6475	2.6798
25	2.0360	2.0584	2.5324	2.1676	2.5489	2.4762	2.5915	2.4748	2.4410	2.6047
30	1.8783	2.0216	2.4791	1.9089	2.5017	2.4051	2.5240	2.4299	2.1976	2.4907
35	1.6954	1.9249	2.4541	1.7301	2.3526	2.3532	2.4372	2.3980	2.0030	2.1973
40	1.6006	1.8215	2.4236	1.5834	2.1519	2.3148	2.3527	2.3852	1.8285	1.8502
45	1.5716	1.6677	2.4093	1.5090	1.9781	2.2308	2.1710	2.3662	1.7168	1.6123

	Set A1					Controls		
Time	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctr	20 μM	40 μM
10	2.8525	2.8139	2.7650	2.8432	2.7920	2.5546	2.4364	2.4642
15	2.7700	2.7270	2.5963	2.7615	2.6862	2.4468	2.3277	2.3485
20	2.6894	2.6471	2.3595	2.6588	2.4871	2.3644	2.2347	2.2544
25	2.5911	2.5560	2.2256	2.5606	2.3163	2.2857	2.1596	2.1762
30	2.5328	2.4766	2.1374	2.4883	2.1206	2.2238	2.1020	2.1120
35	2.4942	2.4069	2.0078	2.4212	2.0250	2.1652	2.0531	2.0654
40	2.4586	2.3628	1.9496	2.3737	1.8596	2.1240	2.0081	2.0246
45	2.4298	2.3118	1.8073	2.3358	1.7551	2.0841	1.9775	1.9914

Table B-104.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 12Dec2011

Time	Set C1					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	2.4330	2.3058	1.8969	2.6797	2.3218	1.9104	2.0018	1.7432	2.6498	2.6475
15	2.4127	2.2243	1.8815	2.6707	2.3026	1.7583	1.9012	1.6942	2.5901	2.5507
20	2.4011	2.0835	1.6601	2.6503	2.2649	1.7410	1.8352	1.5201	2.4644	2.4706
25	2.3299	2.0688	1.5929	2.4998	1.9968	1.6651	1.8177	1.5278	2.3446	2.3169
30	2.2129	1.9547	1.5144	2.3839	1.8660	1.6475	1.7782	1.4417	2.2772	2.2782
35	2.1365	1.9873	1.4131	2.3437	1.8511	1.5552	1.7119	1.3609	2.2291	2.2512
45	1.9836	1.8523	1.3317	2.2152	1.7399	1.5215	1.7196	1.3253	2.1391	2.2494

Time	Set A1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctr	20 μM	40 μM
10	2.0691	1.9179	2.3212	2.0470	2.3351	2.1836	2.2151	2.2379
15	2.0305	1.8561	2.2867	1.9299	2.2707	2.1362	2.1669	2.0909
20	2.0200	1.8328	2.2785	1.9314	2.2525	2.1079	2.1389	2.1661
25	2.0169	1.7945	2.2688	1.8394	2.2411	2.0801	2.1146	2.1455
30	2.0096	1.7623	2.2753	1.8644	2.1719	2.0639	2.1014	2.1292
35	2.0036	1.5322	2.2787	1.8546	2.0906	2.0495	2.0843	2.1189
45	1.9432	1.4415	2.2715	1.8142	1.8487	2.0308	2.0642	2.0987

Table B-104.3c. Batch 3, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 12Dec2011

Time	Set C1					Set C2				
	rxn 1	**rxn 2**	**rxn 3**	**rxn 4**	**rxn 5**	**rxn 6**	**rxn 7**	**rxn 8**	**rxn 9**	**rxn 10**
(min)	**C1-0**	**C1-10**	**C1-20**	**C1-30**	**C1-40**	**C2-0**	**C2-10**	**C2-20**	**C2-30**	**C2-40**
10	2.4517	2.3921	2.6115	2.4544	2.4573	2.4350	2.3619	2.5267	2.4929	2.2603
15	2.4419	2.3820	2.6057	2.4312	2.4247	2.4191	2.3462	2.5151	2.4649	2.2231
20	2.4417	2.3776	2.5848	2.4066	2.3973	2.4219	2.3390	2.5019	2.4537	2.1970
25	2.4337	2.3632	2.5803	2.3860	2.3720	2.4130	2.3280	2.4909	2.4341	2.1649
30	2.4301	2.3499	2.5604	2.3665	2.3380	2.4010	2.3160	2.4730	2.4135	2.1316
35	2.4272	2.3465	2.5474	2.3469	2.3158	2.3983	2.3106	2.4617	2.3980	2.1057
40	2.4210	2.3359	2.5359	2.3313	2.2879	2.3968	2.3007	2.4499	2.3827	2.0809
45	2.4193	2.3271	2.5295	2.3145	2.2660	2.3937	2.2920	2.4336	2.3683	2.0562
50	2.4103	2.3168	2.5182	2.2967	2.2443	2.3862	2.2814	2.4227	2.3482	2.0307
55	2.4049	2.3119	2.4740	2.2897	2.2204	2.3868	2.2725	2.4098	2.3348	2.0084
60	2.4091	2.3019	2.3868	2.2706	2.2021	2.3810	2.2670	2.3993	2.3198	1.9849
Rate[a]	0.0009	0.0018	0.0036	0.0036	0.0051	0.0010	0.0019	0.0026	0.0034	0.0055
r^2	0.9685	0.9935	0.8462	0.9896	0.9955	0.9387	0.9945	0.9986	0.9962	0.9954
Rate – neg ctrl rate	0.0003	0.0012	0.0030	0.0030	0.0045	0.0004	0.0013	0.0020	0.0028	0.0049

Time	Set A1					Controls		
	rxn 11	**rxn 12**	**rxn 13**	**rxn 14**	**rxn 15**	**rxn 16**	**rxn 17**	**rxn 18**
(min)	**A1-0**	**A1-10**	**A1-20**	**A1-30**	**A1-40**	**neg ctr**	**20 μM**	**40 μM**
10	2.2258	2.5352	2.2128	2.3208	2.4849	2.3592	2.3042	2.2861
15	2.2151	2.5210	2.1965	2.2938	2.4564	2.3330	2.2730	2.2339
20	2.2090	2.5246	2.1792	2.2698	2.4298	2.3295	2.2574	2.2039
25	2.2054	2.5135	2.1595	2.2551	2.4047	2.3286	2.2442	2.1744
30	2.1987	2.5003	2.1436	2.2321	2.3818	2.3258	2.2296	2.1466
35	2.1954	2.5032	2.1336	2.2143	2.3581	2.3242	2.2146	2.1209
40	2.1890	2.4869	2.1174	2.1952	2.3384	2.3223	2.2045	2.0945
45	2.1863	2.4827	2.1043	2.1787	2.3135	2.3186	2.1918	2.0708
50	2.1815	2.4797	2.0942	2.1663	2.2979	2.3211	2.1811	2.0483
55	2.1761	2.4663	2.0806	2.1493	2.2781	2.3190	2.1671	2.0259
60	2.1689	2.4646	2.0690	2.1310	2.2611	2.3183	2.1565	2.0037
Rate[a]	0.0010	0.0014	0.0029	0.0037	0.0045	0.0006	0.0028	0.0054
r^2	0.9878	0.9731	0.9927	0.9935	0.9945	0.6403	0.9835	0.9883
Rate – neg ctrl rate	0.0004	0.0008	0.0023	0.0031	0.0039	0.0000	0.0022	0.0048

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.3c-C1, C2, C3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
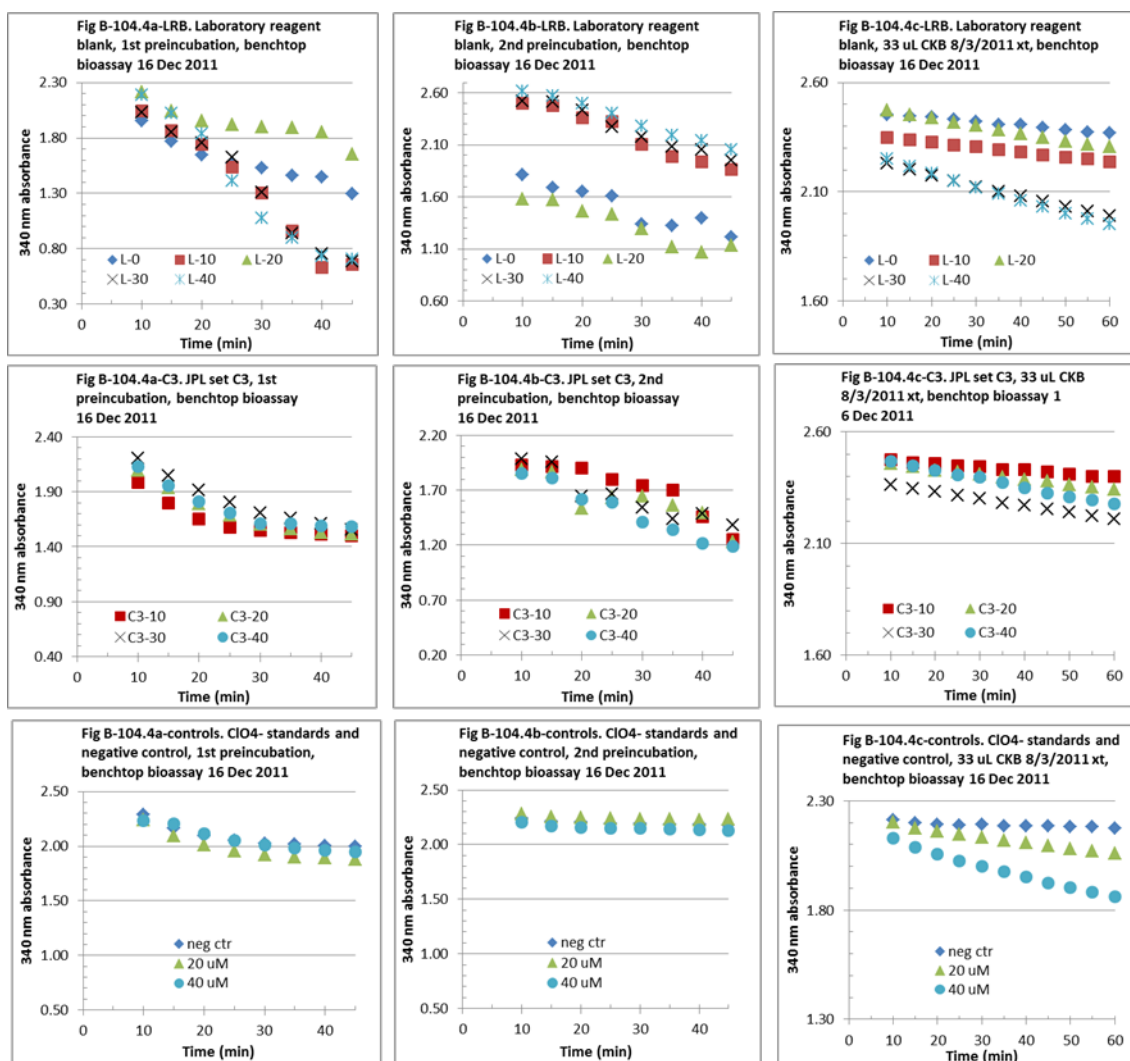
Figure B-104.4. Jet Propulsion Laboratory, benchtop bioassay batch 4, set C3, and Laboratory Reagent Blank set, and perchlorate controls. Test date 16 Dec 2011. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 uL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.4a, b, and c

Data for Figure B-104.4.
Groundwater sample 104, Jet Propulsion Laboratory. Standards addition method benchtop bioassay, batch 4, 16Dec2011. SDVB/DTAB eluate set C3, Laboratory Reagent Blank (L), perchlorate 20 and 40 μM standards, and negative control (no ClO$_4^-$ present).

Table B-104.4a. Batch 4, 340 nm absorbance, 1st preincubation without extract, 16Dec2011

Time	Set L					Set C3			
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9
(min)	L-0	L-10	L-20	L-30	L-40	C3-10	C3-20	C3-30	C3-40
10	1.9544	2.0421	2.2120	2.0305	2.1901	1.9854	2.0940	2.2075	2.1290
15	1.7679	1.8618	2.0423	1.8519	2.0197	1.7956	1.9320	2.0464	1.9578
20	1.6481	1.7451	1.9537	1.7522	1.8375	1.6534	1.7908	1.9126	1.8097
25	1.5697	1.5384	1.9205	1.6237	1.4160	1.5789	1.6849	1.8033	1.7053
30	1.5260	1.3002	1.9003	1.3091	1.0771	1.5464	1.6068	1.7042	1.6120
35	1.4631	0.9601	1.8915	0.9397	0.8985	1.5269	1.5595	1.6567	1.6081
40	1.4499	0.6294	1.8521	0.7524	0.7351	1.5125	1.5308	1.6084	1.5921
45	1.2943	0.6603	1.6557	0.6851	0.7035	1.5024	1.5165	1.5550	1.5800

Time	Controls		
	rxn 10	rxn 11	rxn 12
(min)	neg ctr	20 μM	40 μM
10	2.2915	2.2376	2.2311
15	2.1636	2.0967	2.2066
20	2.0932	2.0105	2.1121
25	2.0532	1.9560	2.0551
30	2.0276	1.9216	2.0128
35	2.0152	1.9016	1.9859
40	2.0073	1.8895	1.9633
45	1.9986	1.8808	1.9460

Table B-104.4b. Batch 4, 340 nm absorbance, 2nd preincubation without extract, 16Dec2011

Time	Set L					Set C3			
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9
(min)	L-0	L-10	L-20	L-30	L-40	C3-10	C3-20	C3-30	C3-40
10	1.8113	2.5006	1.5760	2.5217	2.6174	1.9279	1.8872	1.9809	1.8547
15	1.6865	2.4813	1.5738	2.5144	2.5728	1.9142	1.8632	1.9541	1.8115
20	1.6548	2.3600	1.4608	2.4305	2.4989	1.9001	1.5368	1.6468	1.6182
25	1.6069	2.3214	1.4317	2.2762	2.4062	1.7970	1.6285	1.6621	1.5863
30	1.3385	2.1062	1.2914	2.1781	2.2795	1.7441	1.6424	1.5397	1.4130
35	1.3229	1.9909	1.1178	2.0806	2.1924	1.6986	1.5588	1.4358	1.3418
40	1.3995	1.9386	1.0649	2.0574	2.1409	1.4582	1.4941	1.4889	1.2199
45	1.2155	1.8620	1.1344	1.9548	2.0550	1.2534	1.2297	1.3789	1.1901

Time	Controls		
	rxn 10	rxn 11	rxn 12
(min)	neg ctr	20 μM	40 μM
10	2.2386	2.2794	2.2055
15	2.2122	2.2566	2.1729
20	2.2039	2.2475	2.1574
25	2.1917	2.2363	2.1514
30	2.1940	2.2360	2.1465
35	2.1867	2.2301	2.1393
40	2.1826	2.2282	2.1356
45	2.1794	2.2301	2.1276

Table B-104.4c. Batch 4, 340 nm absorbance, 33 μmL CKB 8/3/2011 extract, 16Dec2011

Time	Set L					Set C3			
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9
(min)	L-0	L-10	L-20	L-30	L-40	C3-10	C3-20	C3-30	C3-40
10	2.4526	2.35	2.4741	2.2302	2.2517	2.4747	2.4575	2.3645	2.4695
15	2.4451	2.3385	2.4534	2.2029	2.2176	2.4622	2.4453	2.347	2.4488
20	2.4423	2.3282	2.4373	2.1745	2.1841	2.4583	2.4281	2.3336	2.4289
25	2.431	2.3134	2.4187	2.1487	2.1498	2.4488	2.4203	2.3142	2.4079
30	2.4202	2.3059	2.4035	2.1236	2.1206	2.4452	2.4103	2.3032	2.3977
35	2.4062	2.2929	2.3841	2.1005	2.0894	2.4306	2.3968	2.2825	2.3727
40	2.4074	2.2808	2.3651	2.0792	2.0592	2.4308	2.3887	2.2715	2.3496
45	2.3935	2.2698	2.3468	2.0564	2.0311	2.4204	2.3803	2.2553	2.3271
50	2.383	2.2592	2.3318	2.0317	2.0019	2.4104	2.3648	2.241	2.3093
55	2.3745	2.2496	2.3159	2.0117	1.9761	2.4016	2.3535	2.2246	2.2961
60	2.3678	2.2376	2.3054	1.991	1.9506	2.3994	2.3426	2.2115	2.2768
Rate[a]	0.0018	0.0022	0.0034	0.0048	0.0060	0.0015	0.0022	0.0031	0.0039
r^2	0.9895	0.9990	0.9976	0.9976	0.9980	0.9889	0.9964	0.0025	0.9972
Rate – neg ctrl rate	0.0012	0.0016	0.0028	0.0042	0.0054	0.0009	0.0016	0.9987	0.0033

Time	Controls		
	rxn 10	rxn 11	rxn 12
(min)	neg ctr	20 μM	40 μM
10	2.2112	2.2017	2.1295
15	2.1977	2.1747	2.0881
20	2.1931	2.1597	2.0575
25	2.1880	2.1459	2.0264
30	2.1914	2.1339	2.0006
35	2.1852	2.1200	1.9752
40	2.1853	2.1068	1.9523
45	2.1833	2.0948	1.9257
50	2.1799	2.0810	1.9020
55	2.1804	2.0709	1.8816
60	2.1754	2.0581	1.8598
Rate[a]	0.0006	0.0027	0.0053
r^2	0.8460	0.9906	0.0047
Rate – neg ctrl rate	0.0000	0.0021	0.9925

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.4c-L, C3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture

Groundwater sample 105, Fontana Water Company, location F-17C, collected 25Jan2012

Figure B-105.1. Fontana Water Company, benchtop bioassay batch 1, sets A3, A2, A1, and perchlorate controls. Test date 16 Feb 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-105.1a, b, and c

Data for Figure B-105.1.
Groundwater sample 105, FWC. Standards addition method benchtop bioassay, batch 1, 16Feb2012. SDVB/DTAB eluate sets A1, A2, A3, perchlorate 20 and 40 µM standards, and negative control (no ClO_4^- present)

Table B-105.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 16Feb2012

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	1.9230	1.8909	1.8462	1.7218	1.7545	1.7499	1.9642	1.7218	2.1005	1.9130
15	1.7900	1.7431	1.6975	1.5639	1.5709	1.5792	1.8058	1.5460	1.9493	1.7612
20	1.6843	1.6517	1.6005	1.4872	1.4638	1.4760	1.6918	1.4412	1.8510	1.6547
25	1.5931	1.5821	1.5336	1.4611	1.3904	1.4124	1.6033	1.3650	1.7899	1.5650
30	1.5244	1.5497	1.5036	1.4126	1.3028	1.3777	1.5515	1.1278	1.7622	1.5235
35	1.4733	1.5324	1.4884	1.3200	1.2529	1.3634	1.2686	0.8452	1.7454	1.4998
40	1.4394	1.5262	1.4802	0.9589	1.1520	1.3524	0.7465	0.6715	1.7294	1.4882
45	1.3979	1.5239	1.4744	0.7450	1.0257	1.2878	0.6274	0.5826	1.7100	1.4780

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 µM	40 µM
10	1.9088	1.9317	1.9496	1.9634	2.0600	2.4179	2.5017	2.4181
15	1.7485	1.7847	1.8172	1.8232	1.9304	2.3207	2.4086	2.3174
20	1.6388	1.6601	1.6969	1.6975	1.8148	2.2424	2.3317	2.2309
25	1.5841	1.5630	1.5914	1.5868	1.7024	2.1993	2.2790	2.1699
30	1.5626	1.5074	1.4920	1.5115	1.6299	2.1659	2.2448	2.1393
35	1.5537	1.4739	1.3190	1.4569	1.5412	2.1450	2.2193	2.1129
40	1.5439	1.4615	0.9605	1.4218	1.4470	2.1321	2.2052	2.1003
45	1.5375	1.4514	0.7966	1.3909	1.1970	2.1246	2.1909	2.0917

Table B-105.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 16Feb2012

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
11.5	1.8327	1.8113	1.5685	2.6006	1.8633	1.7701	2.5433	2.4171	1.5911	1.4909
15	1.8248	1.5879	1.5494	2.5894	1.8084	1.7602	2.5375	2.4190	1.5324	1.4779
20	1.8072	1.4141	1.3483	2.5820	1.7219	1.7188	2.5042	2.4074	1.3400	1.4632
25	1.8009	1.4236	1.4246	2.5731	1.6466	1.4763	2.3184	2.3091	1.2982	1.3344
30	1.7956	1.3859	1.4134	2.5408	1.5808	1.3929	2.1739	2.0362	1.2425	1.1298
35	1.7594	1.2255	1.3404	2.4636	1.5850	1.2667	2.0558	2.0358	1.1926	0.9892
40	1.6507	1.1850	1.2815	2.3672	1.5686	1.2010	1.9351	1.9803	1.0760	0.9375
45	1.3841	1.1645	1.2600	2.0584	1.5048	1.1095	1.8580	1.8765	1.0166	0.9226

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 µM	40 µM
11.5	1.7512	1.6273	2.4048	1.8968	2.0589	2.3324	2.2478	2.1963
15	1.7339	1.5227	2.3768	1.8682	2.0313	2.3126	2.2211	2.1743
20	1.4205	1.2859	2.3627	1.6041	1.9720	2.2965	2.2062	2.1533
25	1.4655	1.2245	2.3257	1.5552	1.7566	2.2745	2.1924	2.1401
30	1.3444	1.1023	2.0791	1.3838	1.6034	2.2771	2.1838	2.1328
35	1.3444	1.0387	1.9545	1.3573	1.4565	2.2701	2.1766	2.1297
40	1.2487	1.0775	1.7965	1.3098	1.5584	2.2688	2.1741	2.1285
45	1.2037	0.9252	1.7518	1.2867	1.4424	2.2617	2.1710	2.1228

Table B-105.1c. Batch 1, 340 nm absorbance, 25 μL CKB 12/10/2007 extract, 16Feb2012

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.1830	2.3346	2.1616	2.2333	2.2320	2.4763	2.3741	2.2134	2.3629	2.3261
15	2.1601	2.3125	2.1175	2.1809	2.1651	2.4571	2.3455	2.1675	2.3022	2.2541
20	2.1441	2.2965	2.0799	2.1418	2.1183	2.4618	2.3305	2.1384	2.2627	2.2047
25	2.1304	2.2793	2.0499	2.1037	2.0710	2.4446	2.3083	2.1089	2.2210	2.1429
30	2.1185	2.2640	2.0222	2.0739	2.0311	2.4410	2.2910	2.0844	2.1964	2.1124
35	2.1044	2.2537	2.0043	2.0519	1.9988	2.4345	2.2826	2.0642	2.1696	2.0826
40	2.1006	2.2455	1.9861	2.0329	1.9773	2.4181	2.2725	2.0513	2.1442	2.0545
45	2.0919	2.2360	1.9755	2.0140	1.9606	2.4243	2.2656	2.0376	2.1286	2.0296
50	2.0860	2.2332	1.9625	2.0034	1.9411	2.4201	2.2591	2.0267	2.1157	2.0166
55	2.0800	2.2271	1.9544	1.9942	1.9287	2.4219	2.2555	2.0184	2.1089	2.0035
60	2.0781	2.2268	1.9472	1.9838	1.9188	2.4238	2.2485	2.0118	2.0967	1.9879
Rate[a]	0.0020	0.0021	0.0041	0.0048	0.0060	0.0011	0.0024	0.0038	0.0051	0.0064
r^2	0.9386	0.9272	0.9266	0.9308	0.9299	0.8326	0.9240	0.9305	0.9281	0.9290
Rate – neg ctrl rate	0.0020	0.0021	0.0041	0.0048	0.0060	0.0011	0.0024	0.0038	0.0051	0.0064

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 μM	40 μM
10	2.3035	2.4874	2.2791	2.3717	2.3500	2.3901	2.3670	2.3449
15	2.2795	2.4560	2.2152	2.3002	2.2634	2.3888	2.3384	2.2938
20	2.2666	2.4419	2.1804	2.2589	2.2108	2.3971	2.3196	2.2561
25	2.2585	2.4200	2.1447	2.2137	2.1627	2.3949	2.3016	2.2211
30	2.2486	2.4055	2.1201	2.1724	2.1288	2.3978	2.2781	2.1832
35	2.2454	2.3966	2.1011	2.1472	2.0961	2.3873	2.2697	2.1509
40	2.2328	2.3878	2.0828	2.1266	2.0725	2.3949	2.2519	2.1218
45	2.2330	2.3748	2.0705	2.1044	2.0571	2.3920	2.2358	2.0963
50	2.2288	2.3610	2.0595	2.0898	2.0415	2.3942	2.2288	2.0750
55	2.2283	2.3671	2.0494	2.0773	2.0258	2.3887	2.2161	2.0546
60	2.2260	2.3632	2.0426	2.0674	2.0216	2.3877	2.2096	2.0333
Rate[a]	0.0014	0.0024	0.0044	0.0058	0.0061	0.00005	0.0031	0.0061
r^2	0.8772	0.9221	0.9077	0.9278	0.9044	0.0551	0.9745	0.9819
Rate – neg ctrl rate	0.0014	0.0024	0.0044	0.0058	0.0061	0.0000	0.0031	0.0061

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-105.1c-A1, A2, A3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
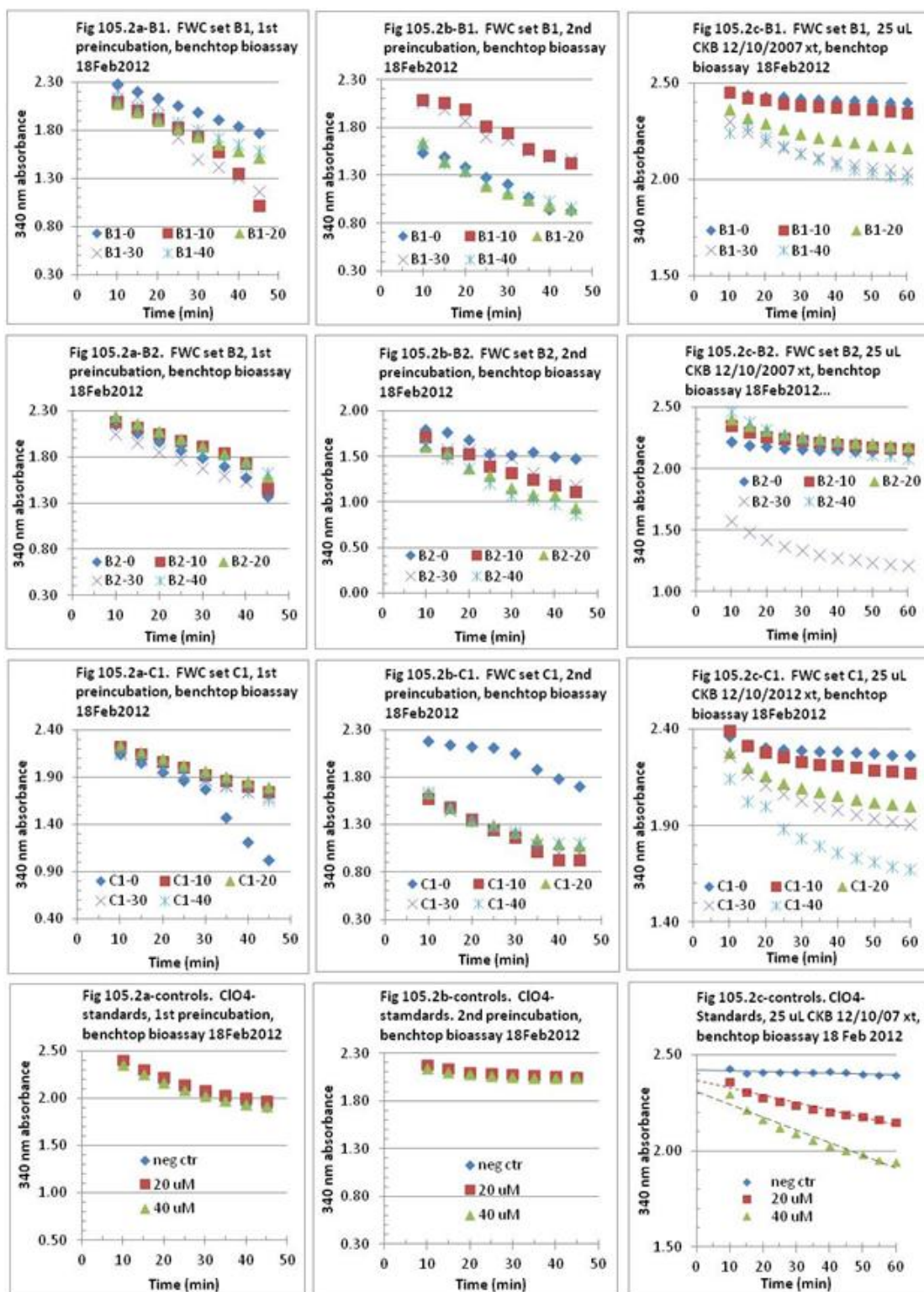
Figure B-105.2. Fontana Water Company, benchtop bioassay batch 1, sets B1, B2, C1, and perchlorate controls. Test date 18 Feb 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-105.2a, b, and c

Data for Figure B-105.2.
Groundwater sample 105, FWC. Standards addition method benchtop bioassay, batch 2,
18Feb2012. SDVB/DTAB eluate sets B1, B2, C1, perchlorate 20 and 40 µM standards, and
negative control (no ClO$_4^-$ present)

Table B-105.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 18Feb2012

	Set B1					Set B2				
Time	**rxn 1**	**rxn 2**	**rxn 3**	**rxn 4**	**rxn 5**	**rxn 6**	**rxn 7**	**rxn 8**	**rxn 9**	**rxn 10**
(min)	**B1-0**	**B1-10**	**B1-20**	**B1-30**	**B1-40**	**B2-0**	**B2-10**	**B2-20**	**B2-30**	**B2-40**
10	2.2801	2.0992	2.0786	2.2084	2.1386	2.1486	2.1762	2.2446	2.0511	2.1678
15	2.2074	2.0096	1.9841	2.1425	2.0498	2.0582	2.1147	2.1624	1.9558	2.0807
20	2.1308	1.9220	1.8945	2.0640	1.9606	1.9641	2.0494	2.0750	1.8516	1.9888
25	2.0524	1.8338	1.8123	1.7263	1.8807	1.8730	1.9766	1.9936	1.7667	1.9099
30	1.9856	1.7351	1.7286	1.4922	1.8031	1.7912	1.9117	1.9120	1.6766	1.8296
35	1.9079	1.5749	1.6529	1.4210	1.7233	1.6950	1.8464	1.8356	1.6005	1.7631
40	1.8404	1.3461	1.5826	1.3057	1.6502	1.5767	1.7329	1.7539	1.5289	1.6903
45	1.7735	1.0163	1.5201	1.1632	1.5843	1.3672	1.4533	1.5895	1.4286	1.6273

	Set C1					Controls		
Time	**rxn 11**	**rxn 12**	**rxn 13**	**rxn 14**	**rxn 15**	**rxn 16**	**rxn 17**	**rxn 18**
(min)	**C1-0**	**C1-10**	**C1-20**	**C1-30**	**C1-40**	**neg ctr**	**20 µM**	**40 µM**
10	2.1373	2.2153	2.2250	2.1598	2.1376	2.3677	2.4107	2.3533
15	2.0459	2.1400	2.1601	2.0848	2.0640	2.2720	2.3080	2.2597
20	1.9434	2.0595	2.0852	2.0059	1.9904	2.1691	2.2264	2.1667
25	1.8544	1.9936	2.0188	1.9369	1.9240	2.0894	2.1483	2.0912
30	1.7625	1.9211	1.9547	1.8673	1.8599	2.0226	2.0827	2.0267
35	1.4699	1.8611	1.9012	1.8050	1.7975	1.9754	2.0381	1.9788
40	1.2101	1.7971	1.8423	1.7416	1.7342	1.9411	2.0035	1.9420
45	1.0200	1.7407	1.7898	1.6904	1.6614	1.9104	1.9726	1.9183

Table B-105.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 18Feb2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	1.5322	2.0822	1.6420	2.0604	1.6076	1.7988	1.7085	1.6089	1.6275	1.6400
15	1.4891	2.0547	1.4480	1.9917	1.4245	1.7661	1.5365	1.5446	1.5808	1.4712
20	1.3840	1.9918	1.3412	1.8712	1.3469	1.6795	1.5254	1.3686	1.5625	1.3703
25	1.2807	1.8125	1.1914	1.7005	1.2294	1.5236	1.3866	1.2903	1.5354	1.2073
30	1.2087	1.7413	1.1053	1.6688	1.1440	1.5126	1.3179	1.1506	1.4861	1.0715
35	1.0721	1.5746	1.0367	1.5487	1.0679	1.5439	1.2434	1.0794	1.3226	1.0267
40	0.9378	1.5071	0.9781	1.4947	1.0254	1.4949	1.1802	1.0697	1.2197	0.9709
45	0.9259	1.4206	0.9543	1.4681	0.9637	1.4724	1.1090	0.9336	1.1883	0.8632

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 μM	40 μM
10	2.1783	1.5741	1.6293	1.6066	1.6373	2.1720	2.1827	2.1407
15	2.1359	1.4820	1.4731	1.4411	1.4789	2.1307	2.1395	2.1026
20	2.1175	1.3514	1.3372	1.3658	1.3335	2.1018	2.1061	2.0803
25	2.1035	1.2412	1.2930	1.2274	1.2648	2.0897	2.0898	2.0646
30	2.0481	1.1585	1.2112	1.1941	1.2235	2.0802	2.0777	2.0544
35	1.8826	1.0105	1.1415	1.0795	1.0945	2.0677	2.0739	2.0465
40	1.7777	0.9267	1.0890	1.0019	1.1064	2.0660	2.0620	2.0418
45	1.7034	0.9210	1.0756	1.0008	1.1006	2.0583	2.0547	2.0374

Table B-105.2c. Batch 2, 340 nm absorbance, 25 μL CKB 12/10/2007 extract, 18Feb2012

Time	Set B1					Set B2				
	rxn 1	**rxn 2**	**rxn 3**	**rxn 4**	**rxn 5**	**rxn 6**	**rxn 7**	**rxn 8**	**rxn 9**	**rxn 10**
(min)	**B1-0**	**B1-10**	**B1-20**	**B1-30**	**B1-40**	**B2-0**	**B2-10**	**B2-20**	**B2-30**	**B2-40**
10	2.4537	2.4517	2.3646	2.2965	2.2396	2.2207	2.3513	2.4011	1.5784	2.4633
15	2.4380	2.4188	2.3170	2.2441	2.2700	2.1923	2.2962	2.3479	1.4854	2.3819
20	2.4235	2.4094	2.2899	2.1953	2.2166	2.1792	2.2602	2.3141	1.4201	2.3186
25	2.4238	2.3921	2.2608	2.1626	2.1717	2.1665	2.2427	2.2852	1.3703	2.2692
30	2.4156	2.3795	2.2365	2.1340	2.1326	2.1587	2.2303	2.2620	1.3347	2.2285
35	2.4107	2.3748	2.2154	2.1119	2.1013	2.1539	2.2155	2.2445	1.3026	2.1875
40	2.4040	2.3729	2.2026	2.0888	2.0719	2.1495	2.2002	2.2299	1.2769	2.1648
45	2.4081	2.3631	2.1898	2.0743	2.0488	2.1418	2.1917	2.2155	1.2572	2.1349
50	2.4056	2.3601	2.1755	2.0606	2.0318	2.1429	2.1761	2.2052	1.2400	2.1165
55	2.3942	2.3542	2.1691	2.0476	2.0124	2.1401	2.1686	2.1907	1.2243	2.1055
60	2.3981	2.3414	2.1605	2.0336	2.0019	2.1327	2.1554	2.1841	1.2136	2.0839
Rate[a]	0.0010	0.0019	0.0039	0.005	0.0056	0.0015	0.0034	0.0040	0.0067	0.0072
r²	0.8697	0.9102	0.9361	0.9375	0.9585	0.8626	0.9173	0.9295	0.9061	0.9339
Rate – neg ctrl rate	0.0006	0.0015	0.0035	0.0046	0.0052	0.0011	0.0030	0.0036	0.0063	0.0068

Time	Set C1					Controls		
	rxn 11	**rxn 12**	**rxn 13**	**rxn 14**	**rxn 15**	**rxn 16**	**rxn 17**	**rxn 18**
(min)	**C1-0**	**C1-10**	**C1-20**	**C1-30**	**C1-40**	**neg ctr**	**20 μM**	**40 μM**
10	2.3629	2.3905	2.2776	2.2568	2.1398	2.4286	2.3626	2.2939
15	2.3147	2.3112	2.1996	2.1631	2.0221	2.4023	2.3067	2.2141
20	2.3024	2.2793	2.1549	2.1075	1.9982	2.4115	2.2785	2.1640
25	2.2925	2.2540	2.1148	2.0624	1.8837	2.4094	2.2554	2.1204
30	2.2870	2.2306	2.0897	2.0284	1.8343	2.4091	2.2348	2.0900
35	2.2828	2.2151	2.0702	1.9984	1.7927	2.4085	2.2187	2.0558
40	2.2836	2.2070	2.0520	1.9771	1.7586	2.4120	2.2035	2.0267
45	2.2771	2.1973	2.0326	1.9530	1.7309	2.4091	2.1894	2.0012
50	2.2722	2.1859	2.0196	1.9366	1.7081	2.3985	2.1764	1.9799
55	2.2634	2.1780	2.0066	1.9194	1.6849	2.3963	2.1614	1.9541
60	2.2606	2.1685	2.0005	1.9065	1.6721	2.3938	2.1486	1.9399
Rate[a]	0.0015	0.0037	0.0050	0.0064	0.0090	0.0004	0.0039	0.0067
r²	0.7818	0.8589	0.9028	0.9168	0.9224	0.5495	0.9473	0.9571
Rate – neg ctrl rate	0.0011	0.0033	0.0046	0.0060	0.0086	0.0000	0.0035	0.0063

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-105.2c-B1, B2, C1, and controls. r² is the correlation coefficient for the corresponding regression line for each reaction mixture
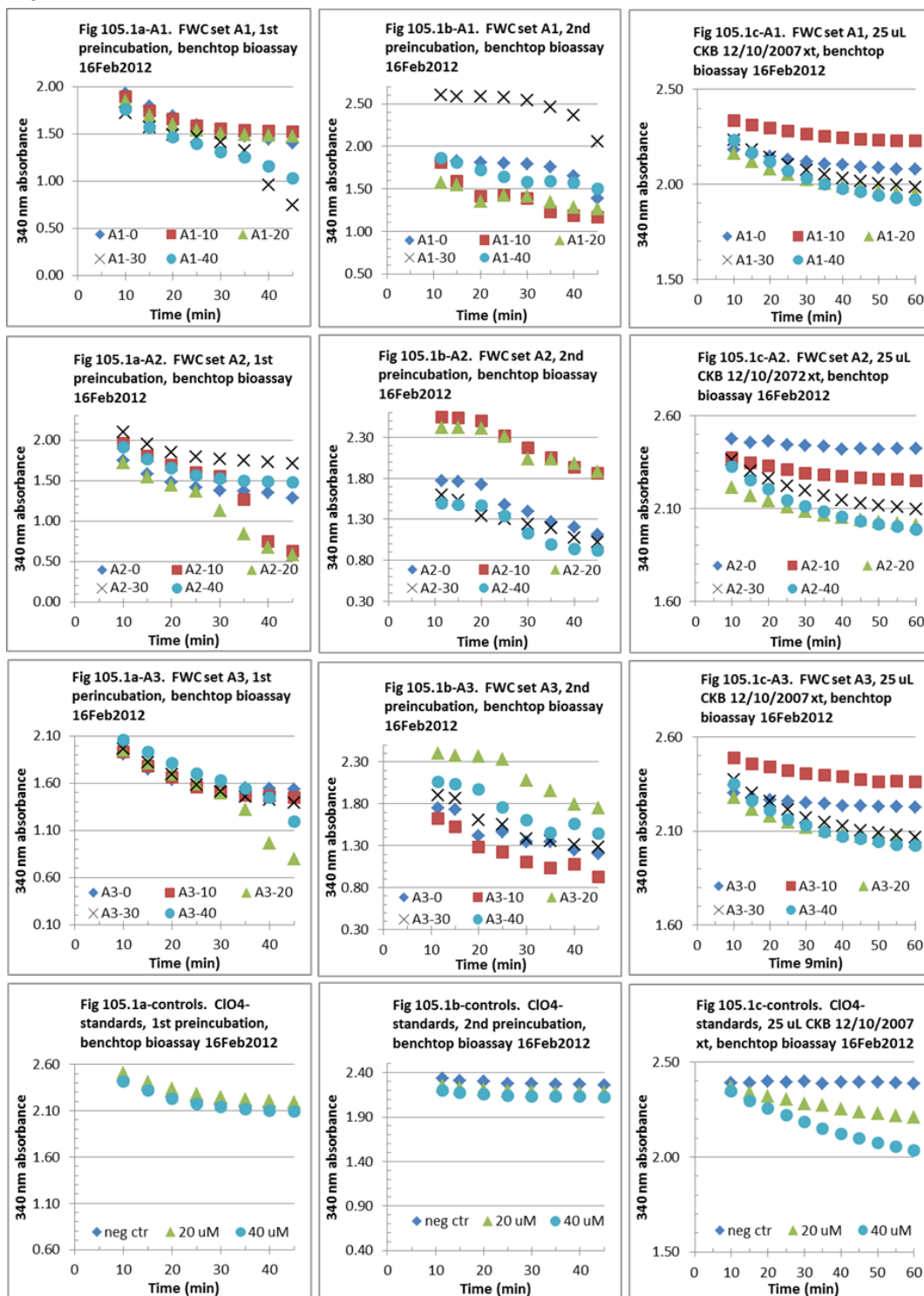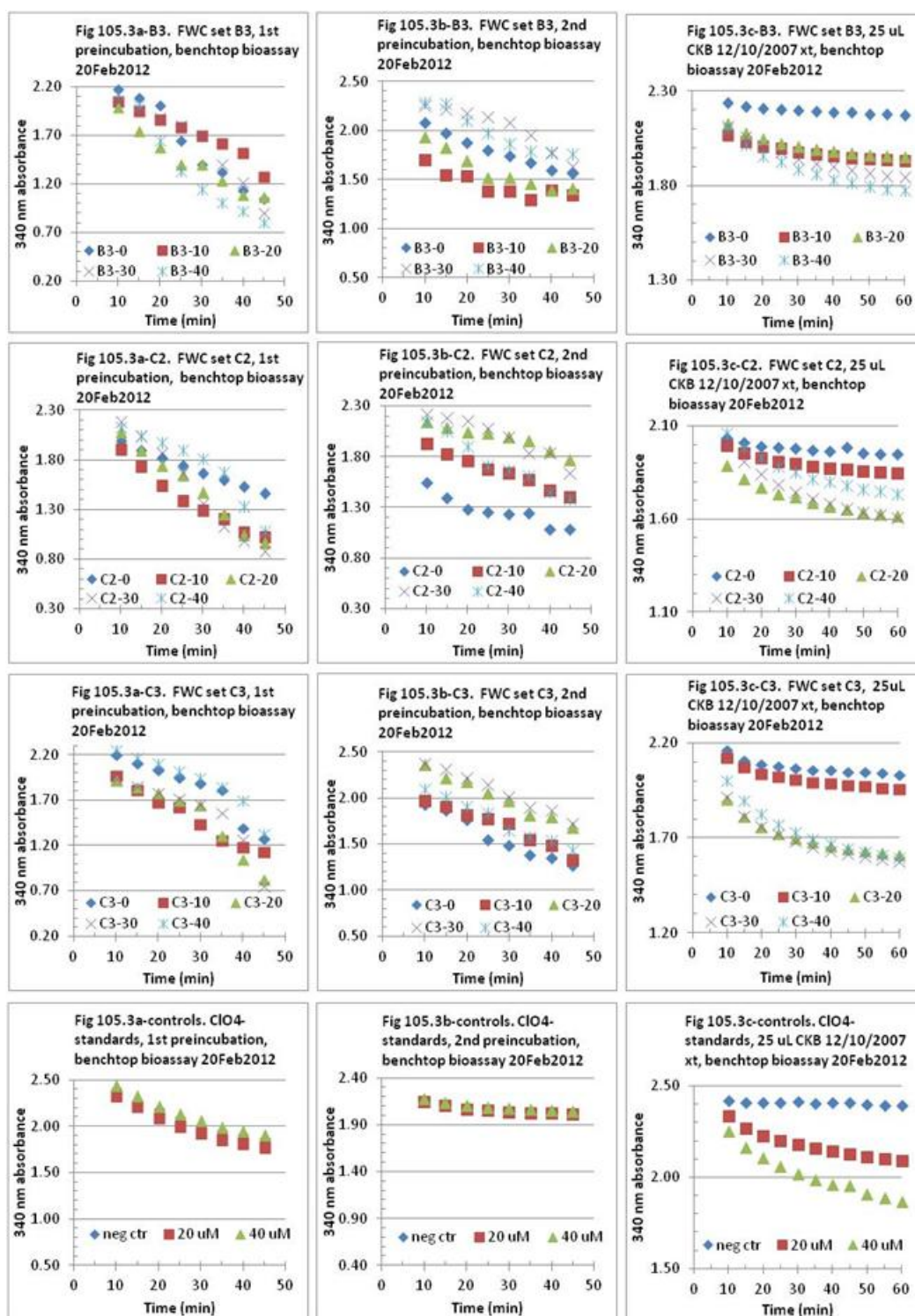
Figure B-105.3. Fontana Water Company, benchtop bioassay batch 3, sets B3, C2, C3, and perchlorate controls. Test date 20 Feb 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-105.3a, b, and c

Data for Figure B-105.3.

Groundwater sample 105, FWC. Standards addition method benchtop bioassay, batch 3, 20Feb2012. SDVB/DTAB eluate sets B3, C2, C3, perchlorate 20 and 40 μM standards, and negative control (no ClO$_4^-$ present)

Table B-105.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 20Feb2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	2.1828	2.0495	1.9912	2.0485	2.1322	1.9906	1.9027	2.0722	2.1856	2.1212
15	2.0955	1.9519	1.7463	1.9700	2.0120	1.8968	1.7329	1.8989	2.0383	2.0357
20	2.0098	1.8675	1.5811	1.8885	1.6519	1.8211	1.5427	1.7435	1.8665	1.9705
25	1.6513	1.7848	1.4013	1.8117	1.3347	1.7452	1.3885	1.6459	1.6627	1.8895
30	1.4025	1.6947	1.4044	1.7040	1.1466	1.6668	1.2888	1.4712	1.3558	1.8070
35	1.3295	1.6165	1.2328	1.4041	1.0083	1.5990	1.2034	1.2527	1.1305	1.6738
40	1.1371	1.5232	1.0893	1.2211	0.9259	1.5319	1.0656	1.0407	0.9831	1.3281
45	1.0533	1.2807	1.0675	0.9060	0.8092	1.4586	1.0168	0.9610	0.8783	1.0753

Time	Set C3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 μM	40 μM
10	2.1981	1.9695	1.9187	1.9349	2.2580	2.3908	2.3186	2.4298
15	2.1104	1.8113	1.8434	1.8516	2.1727	2.2732	2.2059	2.3171
20	2.0325	1.6793	1.7770	1.7807	2.1037	2.1738	2.0874	2.2071
25	1.9540	1.6200	1.6953	1.7168	2.0215	2.0833	1.9967	2.1263
30	1.8816	1.4332	1.6486	1.6537	1.9517	2.0070	1.9188	2.0504
35	1.8091	1.2542	1.3098	1.5655	1.8448	1.9464	1.8519	1.9876
40	1.3919	1.1871	1.0515	1.2716	1.6984	1.8981	1.8036	1.9433
45	1.2746	1.1285	0.8235	0.7484	1.3327	1.8627	1.7641	1.9047

Table B-105.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 20Feb2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	2.0805	1.7026	1.9349	2.2533	2.2802	1.5414	1.9325	2.1465	2.2173	2.1428
15	1.9741	1.5461	1.8200	2.2094	2.2705	1.3962	1.8202	2.0904	2.1822	2.0535
20	1.8706	1.5362	1.6916	2.1734	2.0913	1.2793	1.7613	2.0425	2.1523	1.8999
25	1.7965	1.3742	1.5162	2.1309	1.9743	1.2523	1.6775	2.0269	2.0780	1.7163
30	1.7411	1.3812	1.5152	2.0787	1.8642	1.2334	1.6363	1.9949	1.9949	1.6649
35	1.6729	1.2912	1.4528	1.9537	1.7825	1.2379	1.5702	1.9572	1.8339	1.6120
40	1.5958	1.3895	1.3876	1.7648	1.7770	1.0858	1.4660	1.8495	1.8334	1.4528
45	1.5630	1.3402	1.4085	1.6316	1.7537	1.0829	1.4037	1.7702	1.6398	1.3820

Time	Set C3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 μM	40 μM
10	1.9256	1.9695	2.3624	2.3697	2.0848	2.1651	2.1475	2.1783
15	1.8627	1.9041	2.2165	2.3109	2.0052	2.1152	2.1033	2.1395
20	1.7602	1.8105	2.1690	2.2116	1.8986	2.0817	2.0662	2.1091
25	1.5406	1.7721	2.0434	2.1413	1.8319	2.0622	2.0501	2.0865
30	1.4841	1.7185	1.9666	2.0033	1.6447	2.0480	2.0354	2.0746
35	1.3803	1.5423	1.8072	1.8977	1.5689	2.0416	2.0272	2.0650
40	1.3476	1.4779	1.7882	1.8604	1.5283	2.0333	2.0210	2.0569
45	1.2655	1.3211	1.6788	1.7158	1.4294	2.0285	2.0168	2.0481

Table B-105.3c. Batch 3, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 20Feb2012

Time	Set B3					Set C2				
	rxn 1	**rxn 2**	**rxn 3**	**rxn 4**	**rxn 5**	**rxn 6**	**rxn 7**	**rxn 8**	**rxn 9**	**rxn 10**
(min)	**B3-0**	**B3-10**	**B3-20**	**B3-30**	**B3-40**	**C2-0**	**C2-10**	**C2-20**	**C2-30**	**C2-40**
10	2.2369	2.0671	2.1285	2.1129	2.1002	2.0365	1.9916	1.8854	1.9990	2.0603
15	2.2182	2.0289	2.0775	2.0569	2.0182	2.0075	1.9507	1.8144	1.9053	1.9830
20	2.2066	2.0059	2.0482	2.0113	1.9581	1.9908	1.9285	1.7687	1.8391	1.9260
25	2.2012	1.9938	2.0234	1.9746	1.9238	1.9836	1.9087	1.7317	1.7858	1.8830
30	2.1975	1.9781	2.0047	1.9422	1.8868	1.9760	1.8958	1.7157	1.7427	1.8503
35	2.1917	1.9661	1.9930	1.9164	1.8603	1.9682	1.8826	1.6862	1.7071	1.8169
40	2.1863	1.9537	1.9801	1.8998	1.8308	1.9640	1.8729	1.6667	1.6807	1.7982
45	2.1851	1.9477	1.9720	1.8805	1.8135	1.9826	1.8645	1.6486	1.6577	1.7768
50	2.1775	1.9421	1.9617	1.8668	1.7955	1.9542	1.8562	1.6347	1.6375	1.7590
55	2.1779	1.9355	1.9553	1.8513	1.7797	1.9499	1.8508	1.6233	1.6210	1.7477
60	2.1703	1.9286	1.9501	1.8442	1.7737	1.9461	1.8458	1.6135	1.6059	1.7341
Rate[a]	0.0011	0.0025	0.0031	0.0051	0.0061	0.0015	0.0026	0.0050	0.0073	0.0061
r^2	0.9185	0.9163	0.8938	0.9342	0.9192	0.8231	0.9102	0.9195	0.9197	0.9200
Rate – neg ctrl rate	0.0007	0.0020	0.0028	0.0047	0.0056	0.0010	0.0022	0.0045	0.0069	0.0056

Time	Set C3					Controls		
	rxn 11	**rxn 12**	**rxn 13**	**rxn 14**	**rxn 15**	**rxn 16**	**rxn 17**	**rxn 18**
(min)	**C3-0**	**C3-10**	**C3-20**	**C3-30**	**C3-40**	**neg ctr**	**20 µM**	**40 µM**
10	2.1630	2.1220	1.9014	1.9169	2.0033	2.4183	2.3326	2.2504
15	2.0177	2.0707	1.8132	1.8185	1.8975	2.4075	2.2688	2.1623
20	2.0863	2.0382	1.7579	1.7587	1.8246	2.4080	2.2245	2.1048
25	2.0760	2.0204	1.7229	1.7223	1.7714	2.4064	2.2004	2.0575
30	2.0682	2.0053	1.6959	1.6813	1.7309	2.4118	2.1788	2.0165
35	2.0581	1.9923	1.6739	1.6535	1.6956	2.4007	2.1584	1.9869
40	2.0548	1.9849+	1.6577	1.6304	1.6694	2.4061	2.1430	1.9577
45	2.0479	1.9773	1.6431	1.6138	1.6450	2.4050	2.1259	1.9523
50	2.0449	1.9700	1.6313	1.5990	1.6252	2.3956	2.1097	1.9068
55	2.0398	1.9627	1.6225	1.5875	1.6089	2.3915	2.1000	1.8870
60	2.0336	1.9580	1.6129	1.5781	1.5948	2.3902	2.0903	1.8647
Rate[a]	0.0020	0.0028	0.0051	0.0061	0.0075	0.0005	0.0044	0.0071
r^2	0.7982	0.8643	0.8671	0.8893	0.9068	0.7773	0.9225	0.9460
Rate – neg ctrl rate	0.0016	0.0024	0.0046	0.0057	0.0070	0.0000	0.0039	0.0066

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-105.3c-B3, C2, C3, and controls. r^2 is the correlation c
oefficient for the corresponding regression line for each reaction mixture

Groundwater sample 106, Massachusetts Military Reservation, location J3-INF, collected 23Feb2012

Figure B-106.1. Massachusetts Military Reservation, benchtop bioassay batch 1, sets A1, A2, A3, and perchlorate controls. Test date 15 Mar 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-106.1a, b, and c

Data for Figure B-106.1.
Groundwater sample 106, Massachusetts Military Reservation. Standards addition method benchtop bioassay, batch 1, 15Mar2012. SDVB/DTAB eluate sets A1, A2, A3, perchlorate 20 and 40 μM standards, and negative control (no ClO$_4^-$ present)

Table B-106.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 15Mar2012

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.4409	2.2802	2.2652	2.2844	2.2912	2.3424	2.2320	2.5058	2.3757	2.3686
15	2.4348	2.2253	2.2250	2.2365	2.2499	2.3112	2.1912	2.4794	2.3453	2.3325
20	2.4413	2.1714	2.1918	2.1938	2.2114	2.2804	2.1600	2.4501	2.3097	2.2967
25	2.4457	2.1186	2.1555	2.1472	2.1728	2.2486	2.1274	2.4184	2.2779	2.2598
30	2.4359	2.0608	2.1192	2.0970	2.1354	2.2104	2.0967	2.3877	2.2410	2.2211
35	2.4396	2.0028	2.0796	2.0510	2.0970	2.1777	2.0633	2.3493	2.2008	2.1923
40	2.4194	1.9450	2.0320	2.0061	2.0597	2.1368	2.0324	2.3044	2.1547	2.1404
45	2.4091	1.8938	2.0003	1.9589	2.0096	2.1052	1.9933	2.2813	2.1310	2.1186

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 μM	40 μM
10	2.3553	2.4137	2.3370	2.3784	2.3051	2.4136	2.3371	2.3886
15	2.3111	2.3819	2.2943	2.3418	2.2536	2.3021	2.2249	2.2791
20	2.2833	2.3555	2.2659	2.3126	2.2144	2.2260	2.1342	2.1908
25	2.2473	2.3235	2.2349	2.2835	2.1744	2.1581	2.0642	2.1234
30	2.2109	2.2936	2.2089	2.2565	2.1318	2.1044	2.0135	2.0676
35	2.1725	2.2621	2.1789	2.2179	2.0921	2.0661	1.9659	2.0205
40	2.1369	2.2387	2.1402	2.1846	2.0616	2.0306	1.9328	1.9841
45	2.0911	2.2130	2.1262	2.1586	2.0220	2.0111	1.9123	1.9589

Table B-106.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 15Mar2012

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.0500	1.4545	1.6303	1.5825	1.6484	1.6864	1.5474	1.5291	1.6588	1.7001
15	2.0528	1.3738	1.5401	1.5123	1.6152	1.5897	1.5146	1.4974	1.6247	1.6661
20	1.9255	1.2801	1.4813	1.3561	1.5893	1.5258	1.3665	1.3741	1.5943	1.5628
25	1.8878	1.2334	1.3615	1.3009	1.4836	1.3756	1.3458	1.3629	1.5475	1.4565
30	1.8327	1.1534	1.3678	1.2669	1.3636	1.3421	1.2142	1.1938	1.4897	1.3685
35	1.9150	1.1123	1.2865	1.0862	1.2183	1.2888	1.0962	1.1629	1.3873	1.3195
40	1.8487	1.0734	1.3391	1.0625	1.1415	1.2672	1.0724	1.0442	1.1744	1.2330
45	1.7884	0.9804	1.2682	1.0007	1.0288	1.1442	0.9455	1.0489	0.9845	1.2260

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 µM	40 µM
10	2.2102	1.6194	1.6533	1.6677	1.5550	2.0961	2.1251	2.0771
15	2.1836	1.4683	1.5293	1.5649	1.5082	2.0625	2.0923	2.0472
20	2.1513	1.4218	1.4880	1.5288	1.4197	2.0378	2.0679	2.0185
25	2.0683	1.3487	1.4712	1.3435	1.2589	2.0241	2.0593	1.9994
30	2.0214	1.3151	1.4590	1.3842	1.1331	2.0123	2.0496	1.9906
35	1.9566	1.2655	1.4208	1.2769	1.1339	2.0120	2.0486	1.9869
40	1.9006	1.2313	1.3795	1.2983	1.0275	1.9993	2.0366	1.9823
45	1.7525	1.1989	1.2813	1.2061	0.9601	2.0070	2.0329	1.9841

Table B-106.1c. Batch 1, 340 nm absorbance, 25 μL CKB 12/10/2007 extract, 15Mar2012

Time	Set A1					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.3866	2.1112	1.7855	2.1574	2.1916	2.1053	2.1148	2.1229	2.1751	1.8475
15	2.3894	2.0698	1.6545	2.0516	2.0505	1.9950	1.9899	1.9864	2.0375	1.6824
20	2.3848	2.0490	1.5626	2.0040	1.9426	1.9184	1.8957	1.8732	1.9212	1.5495
25	2.3915	2.0475	1.4974	1.9616	1.8597	1.8703	1.8237	1.7905	1.8441	1.4527
30	2.4003	2.0288	1.4592	1.9292	1.7986	1.8496	1.7773	1.7387	1.7906	1.3846
35	2.3905	2.0235	1.4329	1.9030	1.7540	1.8426	1.7508	1.7083	1.7545	1.3347
40	2.3862	2.0213	1.4165	1.8853	1.7226	1.8375	1.7309	1.6877	1.7278	1.2982
45	2.3853	2.0132	1.4075	1.8636	1.6982	1.8399	1.7249	1.6759	1.7048	1.2734
50	2.3930	2.0016	1.3972	1.8500	1.6832	1.8380	1.7232	1.6620	1.6862	1.2499
55	2.3898	1.9988	1.3906	1.8378	1.6695	1.8394	1.7200	1.6577	1.6764	1.2360
60	2.3714	1.9977	1.3847	1.8287	1.6500	1.8309	1.7095	1.6456	1.6637	1.2160
Rate[a]	0.00014	0.00195	0.00687	0.00582	0.00984	0.00420	0.00703	0.00839	0.00918	0.01143
r^2	0.1062	0.8689	0.7832	0.8860	0.8658	0.6339	0.7710	0.8000	0.8455	0.8598
Rate – neg ctrl rate	-0.00083	0.00098	0.00590	0.00485	0.00886	0.00323	0.00606	0.00742	0.00821	0.01046

Time	Set A3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	neg ctr	20 μM	40 μM
10	1.8868	1.9595	1.8746	1.8974	1.9685	2.4090	2.3824	2.2540
15	1.7777	1.8238	1.7123	1.7245	1.8053	2.4006	2.3451	2.1963
20	1.7005	1.7151	1.5819	1.5856	1.6887	2.4099	2.3268	2.1540
25	1.6500	1.6265	1.4828	1.4777	1.6057	2.4091	2.3021	2.1131
30	1.6188	1.5587	1.4073	1.3966	1.5488	2.3926	2.2916	2.0792
35	1.6059	1.5084	1.3579	1.3410	1.5064	2.3831	2.2743	2.0466
40	1.6008	1.4729	1.3231	1.3046	1.4784	2.3923	2.2478	2.0195
45	1.5943	1.4455	1.3010	1.2785	1.4535	2.3629	2.2282	1.9956
50	1.5930	1.4313	1.2882	1.2582	1.4345	2.3680	2.2370	1.9721
55	1.5904	1.4229	1.2816	1.2458	1.4186	2.3572	2.2178	1.9449
60	1.5943	1.4138	1.2725	1.2355	1.4044	2.3808	2.2195	1.9380
Rate[a]	0.00484	0.01024	0.01102	0.01218	0.01001	0.00097	0.00325	0.00623
r^2	0.69411	0.8646	0.8315	0.84826	0.8488	0.7138	0.9443	0.9718
Rate – neg ctrl rate	0.00387	0.00927	0.01005	0.01121	0.00904	0.00000	0.00228	0.00526

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-106.1c-A1, A2, A3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
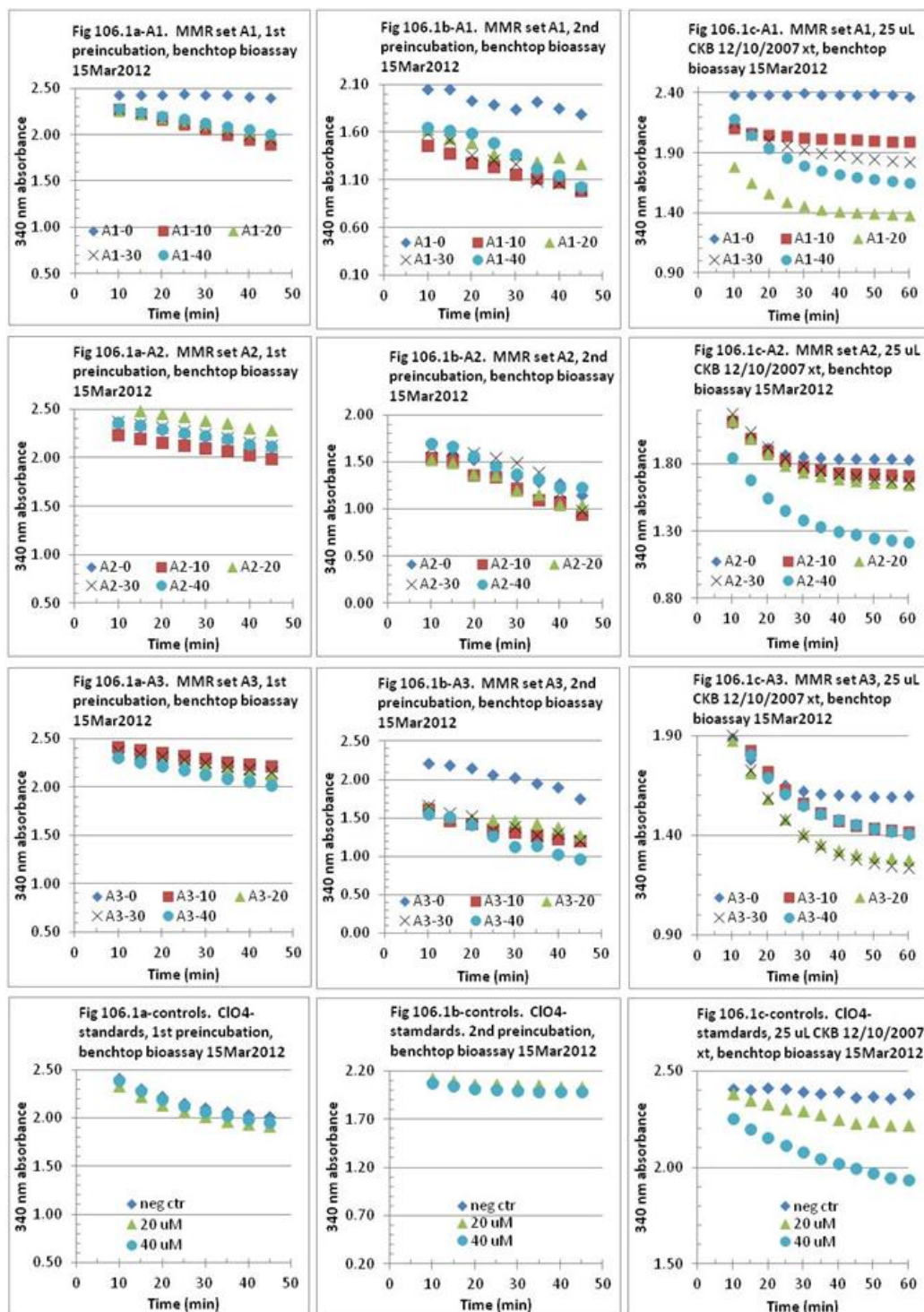
Figure B-106.2. Massachusetts Military Reservation, benchtop bioassay batch 2, sets B1, B2, C1, and perchlorate controls. Test date 18 Mar 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-106.2a, b, and c

Data for Figure B-106.2.
Groundwater sample 106, Massachusetts Military Reservation. Standards addition method
benchtop bioassay, batch 2, 18Mar2012. SDVB/DTAB eluate sets B1, B2, C1, perchlorate 20
and 40 μM standards, and negative control (no ClO$_4^-$ present)

Table B-106.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 18Mar2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.2103	2.3737	2.3161	2.4108	2.4061	2.3494	2.3720	2.3991	2.4405	2.4131
15	2.1718	2.3530	2.1530	2.3783	2.3149	2.3603	2.3760	2.4153	2.3720	2.3325
20	2.0213	2.3367	2.1268	2.3577	2.2679	2.2815	2.3402	2.3608	2.4015	2.3404
25	1.8963	2.3222	1.9861	2.3348	2.0620	2.2419	1.9416	2.3493	2.3867	2.2357
30	1.7669	2.3023	1.9534	2.3127	2.0036	2.1042	1.9063	2.3271	2.3613	2.0887
35	1.6768	2.2759	1.9178	2.2715	2.0256	1.9021	1.8170	2.3143	2.2938	1.9290
40	1.6519	2.2056	1.9002	1.9705	1.9637	1.6088	1.7299	2.3042	2.0178	1.7667
45	1.5837	2.2300	1.8933	1.7330	1.9038	1.5274	1.6758	2.2764	1.7263	1.7568

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 μM	40 μM
10	2.3576	2.4963	2.3816	2.4218	2.5006	2.3096	2.3176	2.3385
15	2.4735	2.3558	2.3990	2.4606	2.4602	2.1773	2.1740	2.1984
20	2.3181	2.4614	2.3348	2.3833	2.4478	2.0835	2.0739	2.1047
25	2.3005	2.4603	2.3163	2.3685	2.4338	2.0192	1.9978	2.0346
30	2.2900	2.4445	2.2582	2.3540	2.4084	1.9714	1.9464	1.9918
35	2.2759	2.4386	2.0024	2.2283	1.9904	1.9341	1.9092	1.9572
40	2.2587	2.4148	1.6685	2.1155	1.8788	1.9124	1.8803	1.9316
45	2.2215	2.0694	1.5593	1.7562	1.8092	1.8901	1.8589	1.9077

Table B-106.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 18Mar2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.2789	1.7322	2.2361	2.2664	2.2185	3.1301	2.3090	1.8634	2.3804	2.2875
15	2.2347	1.6866	2.1710	2.2308	2.0802	3.1447	2.2851	1.8206	2.3648	2.2439
20	2.1072	1.6175	2.1500	2.1596	2.0086	3.1670	2.1929	1.7592	2.2884	2.1927
25	2.1027	1.6077	2.0107	2.0800	1.9995	3.1756	2.1250	1.7586	2.2355	2.1045
30	2.0393	1.5927	1.9785	2.0227	1.9404	3.1572	2.0380	1.6757	2.1060	2.0154
35	2.0074	1.5202	1.9616	1.9385	1.9148	3.0999	1.9717	1.6126	2.0328	2.0101
40	1.9569	1.4526	1.9268	1.9466	1.8773	3.1722	1.9365	1.6136	2.0390	1.9638
45	1.9045	1.4553	1.8843	1.8859	1.8589	3.1078	1.9025	1.5458	1.9749	1.9540

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 μM	40 μM
10	1.9513	2.2224	2.4707	2.3237	2.3434	2.2069	2.1763	2.2239
15	1.8229	2.1063	2.3756	2.2491	2.2840	2.1607	2.1275	2.1925
20	1.7945	2.0561	2.3459	2.1962	2.2760	2.1336	2.1035	2.1755
25	1.7269	1.9974	2.3028	2.1585	2.2036	2.1212	2.0861	2.1627
30	1.6986	1.9383	2.2336	2.1010	2.1692	2.1085	2.0722	2.1476
35	1.6053	1.9105	2.2127	2.0791	2.1332	2.1032	2.0720	2.1442
40	1.5320	1.9470	2.1548	2.0775	2.0600	2.0903	2.0669	2.1407
45	1.5553	1.8963	2.1362	2.0438	2.0564	2.0876	2.0600	2.1348

Table B-106.2c. Batch 2, 340 nm absorbance, 25 μL CKB 12/10/2007 extract, 18Mar2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	1.8896	1.8630	1.8578	1.9093	1.7359	3.0142	2.0237	2.0036	1.9492	1.8108
15	1.7375	1.7036	1.6894	1.7220	1.5476	2.9676	1.8754	1.8314	1.7825	1.6311
20	1.6237	1.5748	1.5510	1.5670	1.4006	2.9207	1.7548	1.7141	1.6482	1.4879
25	1.5506	1.4725	1.4365	1.4403	1.2756	2.8933	1.6542	1.5904	1.5348	1.3664
30	1.5077	1.3883	1.3452	1.3376	1.1747	2.8720	1.5761	1.5011	1.4437	1.2656
35	1.4895	1.3225	1.2742	1.2703	1.0904	2.8670	1.5034	1.4252	1.3620	1.1803
40	1.4763	1.2791	1.2169	1.2098	1.0195	2.8646	1.4512	1.3624	1.2979	1.1101
45	1.4745	1.2395	1.1785	1.1588	0.9584	2.8409	1.4000	1.3066	1.2398	1.0446
50	1.4692	1.2185	1.1515	1.1221	0.9076	2.7903	1.3589	1.2608	1.1926	0.9920
55	1.4638	1.1981	1.1288	1.0917	0.8644	2.8523	1.3205	1.2241	1.1467	0.9471
60	1.4623	1.1889	1.1180	1.0687	0.8247	2.8341	1.2889	1.1914	1.1099	0.9025
Rate[a]	0.0071	0.0128	0.0142	0.0159	0.0174	0.0034	0.0140	0.0156	0.0161	0.0174
r^2	0.7073	0.8879	0.8955	0.9077	0.9415	0.7845	0.9437	0.9411	0.9478	0.9495
Rate – neg ctrl rate	0.0068	0.0125	0.0139	0.0156	0.0171	0.0031	0.0137	0.0153	0.0158	0.0171

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 μM	40 μM
10	2.0208	1.9931	1.9304	1.8600	1.8218	2.5001	2.4122	2.3888
15	1.8620	1.8213	1.7345	1.6475	1.5832	2.4954	2.3699	2.3182
20	1.7396	1.6906	1.5790	1.4819	1.4027	2.5024	2.3460	2.2718
25	1.6344	1.5762	1.4520	1.3463	1.2512	2.4941	2.3237	2.2291
30	1.5495	1.4874	1.3486	1.2382	1.1316	2.5103	2.2840	2.1735
35	1.4809	1.4108	1.2654	1.1464	1.0297	2.4815	2.2838	2.1387
40	1.4278	1.3449	1.1944	1.0695	0.9482	2.5055	2.2552	2.1198
45	1.3829	1.2896	1.1353	1.0028	0.8779	2.4783	2.2367	2.0833
50	1.3456	1.2448	1.0866	0.9511	0.8224	2.4836	2.2279	2.0549
55	1.3165	1.2023	1.0466	0.9038	0.7729	2.4939	2.2180	2.0370
60	1.2943	1.1669	1.0115	0.8646	0.7338	2.4896	2.2081	2.0136
Rate[a]	0.0139	0.0157	0.0175	0.0189	0.0206	0.0003	0.0040	0.0073
r^2	0.9241	0.9431	0.9324	0.9362	0.9309	0.2005	0.9560	0.9684
Rate – neg ctrl rate	0.0136	0.0154	0.0172	0.0186	0.0203	0.0000	0.0037	0.0070

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-106.2c-B1, B2, C1, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
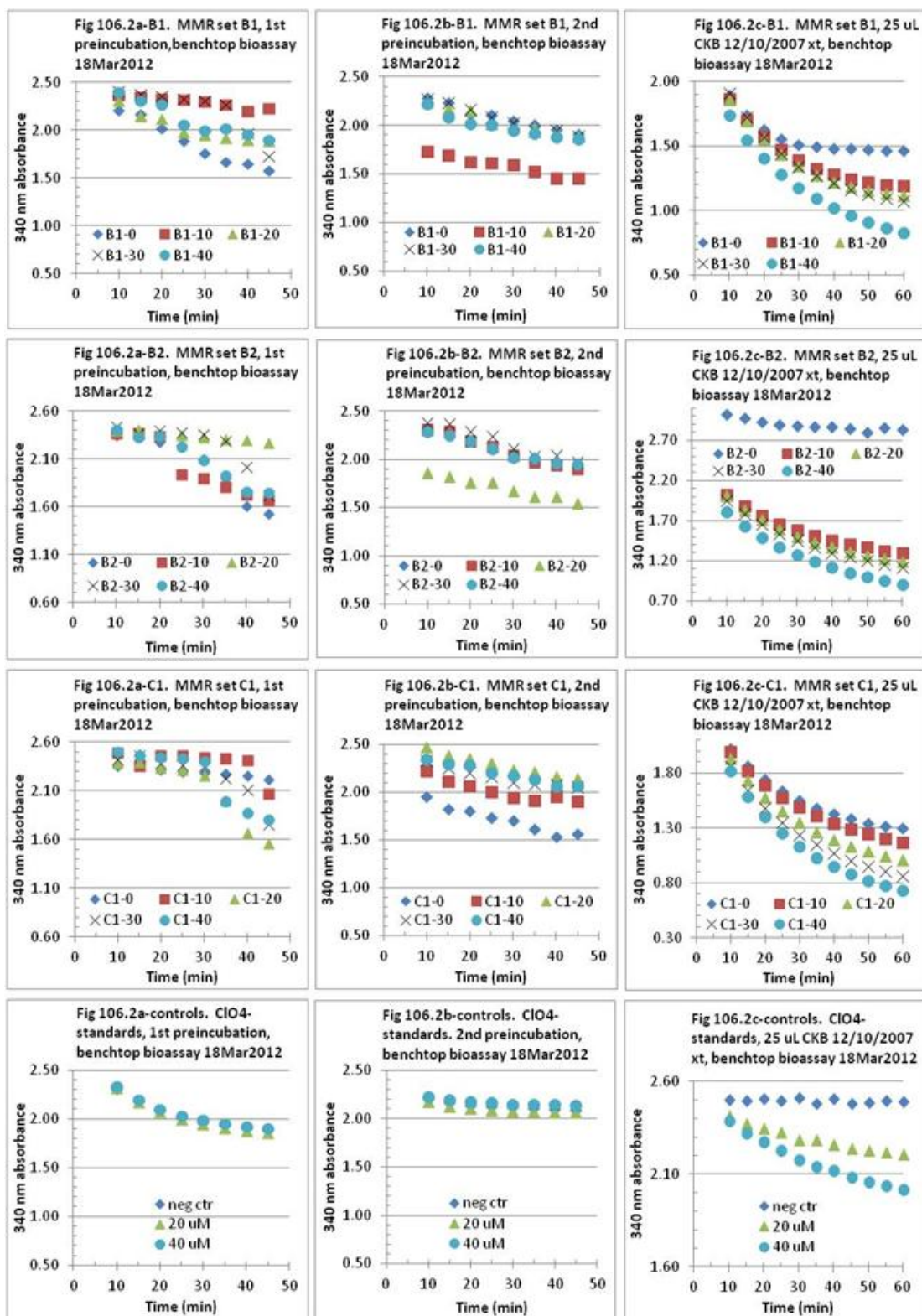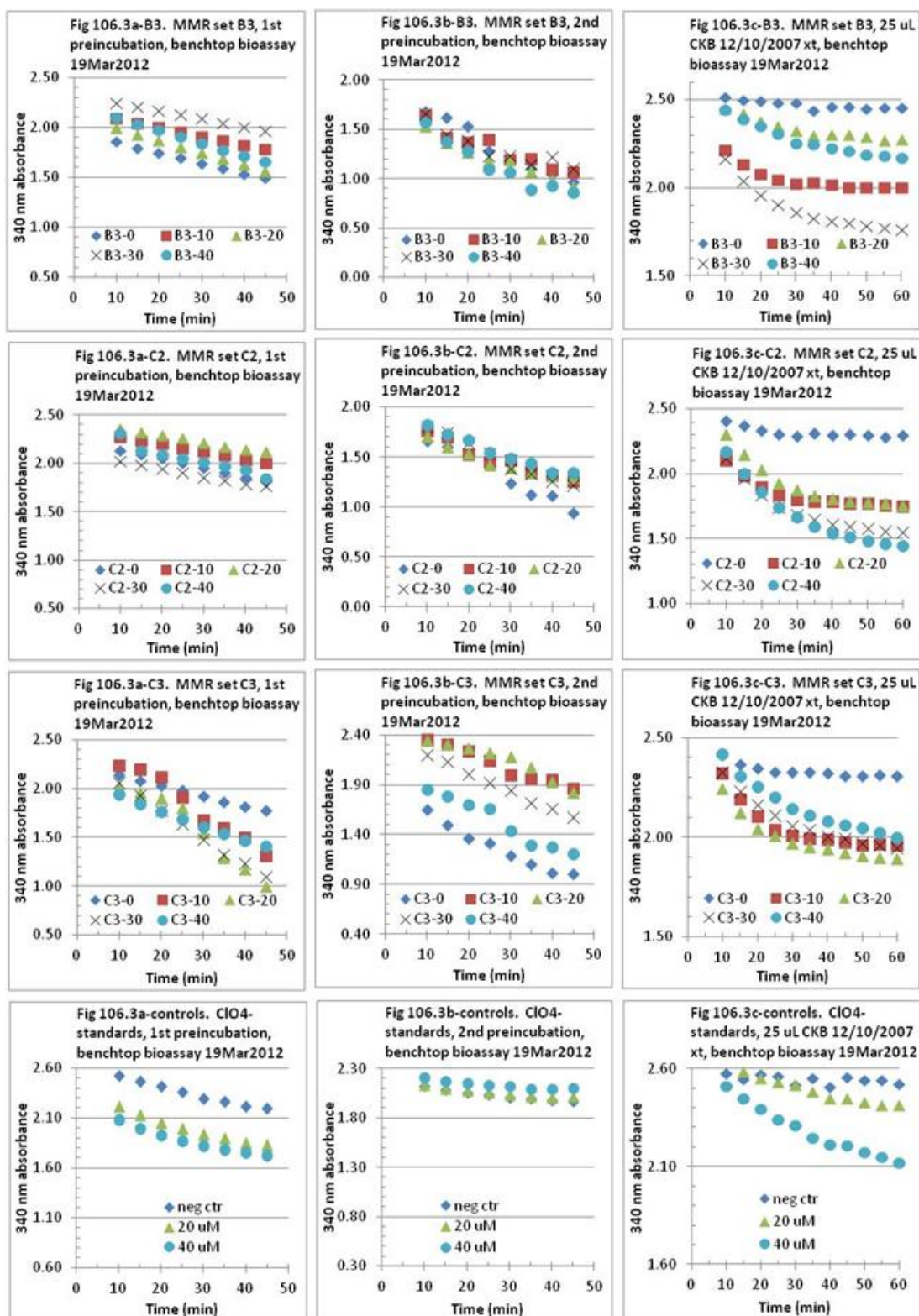
Figure B-106.3. Massachusetts Military Reservation, benchtop bioassay batch 3, sets B3, C2, C3, and perchlorate controls. Test date 19 Mar 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-106.3a, b, and c

Data for Figure B-106.3.
Groundwater sample 106, Massachusetts Military Reservation. Standards addition method benchtop bioassay, batch 3, 19Mar2012. SDVB/DTAB eluate sets B3, C2, C3, perchlorate 20 and 40 μM standards, and negative control (no ClO$_4^-$ present)

Table B-106.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 19Mar2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	1.8629	2.0898	1.9954	2.2470	2.1055	2.1376	2.2721	2.3612	2.0295	2.3176
15	1.7995	2.0445	1.9267	2.2092	2.0330	2.0906	2.2326	2.3268	1.9847	2.1341
20	1.7471	2.0018	1.8704	2.1721	1.9729	2.0521	2.1996	2.2915	1.9453	2.0972
25	1.6948	1.9611	1.8071	2.1283	1.9100	2.0026	2.1589	2.2621	1.9050	2.0601
30	1.6391	1.9126	1.7506	2.0890	1.8386	1.9541	2.1237	2.2124	1.8600	2.0113
35	1.5887	1.8717	1.6904	2.0451	1.7740	1.9041	2.0858	2.1776	1.8315	1.9754
40	1.5377	1.8256	1.6346	2.0066	1.7205	1.8581	2.0383	2.1405	1.7880	1.9404
45	1.4930	1.7863	1.5685	1.9671	1.6629	1.8184	2.0043	2.1294	1.7635	1.8482

Time	Set C3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 μM	40 μM
10	2.1390	2.2406	2.0143	2.0577	1.9434	2.5247	2.2171	2.0814
15	2.0836	2.1938	1.9568	1.9305	1.8461	2.4663	2.1320	2.0024
20	2.0293	2.1151	1.8995	1.7718	1.7658	2.4213	2.0604	1.9359
25	1.9792	1.9108	1.8053	1.6328	1.6832	2.3626	1.9971	1.8752
30	1.9251	1.6773	1.5471	1.4830	1.6024	2.2948	1.9365	1.8239
35	1.8628	1.5996	1.2884	1.3178	1.5337	2.2730	1.9015	1.7874
40	1.8184	1.4992	1.1714	1.2374	1.4677	2.2173	1.8575	1.7612
45	1.7724	1.3147	1.0020	1.0969	1.4118	2.2011	1.8363	1.7267

Table B-106.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 19Mar2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	1.6760	1.6379	1.5307	1.6630	1.5667	1.6609	1.7577	1.7024	1.7959	1.8150
15	1.6241	1.4186	1.3711	1.4451	1.3805	1.5983	1.6961	1.5951	1.7422	1.7209
20	1.5299	1.3663	1.2683	1.3854	1.2845	1.5323	1.5237	1.5325	1.6338	1.6699
25	1.2773	1.3984	1.1865	1.2276	1.0966	1.4292	1.4459	1.4181	1.5293	1.5436
30	1.1792	1.1972	1.1899	1.2264	1.0663	1.2352	1.4615	1.3891	1.3741	1.4830
35	1.1040	1.1973	1.0764	1.1413	0.8955	1.1226	1.3448	1.3389	1.3297	1.4326
40	1.0442	1.0984	0.9848	1.2253	0.9340	1.1063	1.3142	1.3128	1.2551	1.3437
45	0.9707	1.0657	0.9226	1.1042	0.8633	0.9404	1.2576	1.2896	1.2156	1.3403

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 μM	40 μM
10	1.6446	2.3520	2.3380	2.1925	1.8495	2.1270	2.1383	2.2108
15	1.4947	2.2982	2.3025	2.1244	1.7775	2.0896	2.0940	2.1802
20	1.3535	2.2350	2.2636	2.0018	1.6926	2.0572	2.0752	2.1555
25	1.3092	2.1418	2.2173	1.9187	1.6549	2.0361	2.0583	2.1397
30	1.1892	1.9915	2.1761	1.8396	1.4311	2.0108	2.0364	2.1282
35	1.0992	1.9510	2.0714	1.7168	1.2894	2.0024	2.0230	2.1026
40	1.0092	1.9478	1.9302	1.6589	1.2731	1.9835	2.0145	2.1009
45	1.0019	1.8600	1.8202	1.5715	1.2005	1.9669	2.0155	2.1103

Table B-106.3c. Batch 3, 340 nm absorbance, 25 μL CKB 12/10/2007 extract, 19Mar2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	2.5139	2.2167	2.4512	2.1673	2.4452	2.4058	2.1040	2.3013	2.1170	2.1716
15	2.4978	2.1364	2.4216	2.0434	2.3896	2.3660	1.9832	2.1478	1.9617	2.0020
20	2.4934	2.0782	2.3782	1.9594	2.3518	2.3282	1.8972	2.0265	1.8379	1.8610
25	2.4811	2.0468	2.3502	1.9030	2.3071	2.2989	1.8376	1.9218	1.7405	1.7384
30	2.4836	2.0260	2.3222	1.8617	2.2522	2.2845	1.8008	1.8714	1.6777	1.6623
35	2.4367	2.0287	2.2987	1.8271	2.2476	2.3068	1.7865	1.8280	1.6399	1.5949
40	2.4613	2.0167	2.3036	1.8146	2.2282	2.2969	1.7805	1.8040	1.6079	1.5419
45	2.4612	2.0052	2.3030	1.8000	2.2111	2.3025	1.7721	1.7852	1.5903	1.5080
50	2.4494	2.0019	2.2942	1.7871	2.1893	2.2946	1.7659	1.7750	1.5742	1.4839
55	2.4568	2.0031	2.2721	1.7757	2.1810	2.2795	1.7557	1.7697	1.5532	1.4593
60	2.4568	2.0006	2.2732	1.7619	2.1718	2.2979	1.7475	1.7542	1.5435	1.4449
Rate[a]	0.0012	0.0035	0.0034	0.0070	0.0053	0.0018	0.0059	0.0097	0.0103	0.0137
r^2	0.6789	0.7220	0.8669	0.8328	0.9223	0.5768	0.7493	0.8097	0.8386	0.8854
Rate – neg ctrl rate	0.0005	0.0028	0.0027	0.0063	0.0046	0.0011	0.0052	0.0090	0.0096	0.0130

Time	Set C3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 μM	40 μM
10	2.4128	2.3200	2.2415	2.3251	2.4162	2.5742	2.6104	2.5141
15	2.3620	2.1891	2.1243	2.2269	2.3080	2.5447	2.5850	2.4477
20	2.3442	2.1015	2.0399	2.1617	2.2531	2.5709	2.5532	2.3974
25	2.3274	2.0351	2.0058	2.1073	2.2018	2.5613	2.5319	2.3430
30	2.3280	2.0093	1.9687	2.0531	2.1418	2.5176	2.5162	2.3096
35	2.3247	1.9928	1.9517	2.0351	2.1065	2.5522	2.4817	2.2486
40	2.3216	1.9865	1.9418	2.0034	2.0808	2.5062	2.4495	2.2151
45	2.3060	1.9740	1.9199	1.9925	2.0592	2.5560	2.4474	2.2112
50	2.3065	1.9613	1.9070	1.9679	2.0447	2.5439	2.4305	2.1767
55	2.3108	1.9646	1.8952	1.9573	2.0239	2.5418	2.4140	2.1521
60	2.3080	1.9572	1.8896	1.9440	1.9969	2.5206	2.4128	2.1213
Rate[a]	0.0016	0.0060	0.0060	0.0070	0.0076	0.0007	0.0041	0.0076
r^2	0.7128	0.7325	0.8176	0.8932	0.9207	0.2760	0.9690	0.9658
Rate – neg ctrl rate	0.0009	0.0053	0.0053	0.0063	0.0069	0.0000	0.0034	0.0069

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-106.3c-B3, C2, C3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture

Groundwater sample 107, Lawrence Livermore National Laboratory-site 300, location W-854-1823, collected 29 March 2012

Figure B-107.1. Lawrence Livermore National Laboratory-site 300, batch 1, sets B1, B2, C1, and perchlorate controls. Test date 20 Apr 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 (**c**). Data values displayed are listed in Tables B-107.1a, b, and c

Data for Figure B-107.1.

Groundwater sample 107, LLNL-site 300. Standards addition method benchtop bioassay, batch 1, 20April2012: SDVB/DTAB eluate sets B1, B2, C1, perchlorate 20 and 40 µM standards, and negative control (no ClO₄⁻ present)

Table B-107.1a. Batch 1, 340 nm absorbance, 1ˢᵗ preincubation without extract, 20April2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	1.6840	1.4191	1.5650	1.5357	1.3548	1.6318	1.4263	1.6015	1.3934	1.7087
15	1.6609	1.3451	1.5426	1.5003	0.9903	1.5195	1.2314	1.4463	1.3769	1.6782
20	1.6446	1.3291	1.3050	1.4750	0.8296	1.4943	1.1146	1.4132	1.3621	1.6690
25	1.6346	1.3201	1.5250	1.3479	0.8789	1.4455	0.9506	1.1711	1.3541	1.6544
30	1.6277	1.3135	1.5202	1.0839	0.8529	1.2461	0.8509	1.0072	1.3455	1.6546
35	1.6222	1.3046	1.5114	0.9625	0.7545	1.1538	0.8261	0.8586	1.3390	1.6397
40	1.6179	1.2999	1.5073	0.7694	0.7026	0.9419	0.8056	0.7105	1.3370	1.5929
45	1.6108	1.2952	1.5025	0.6672	0.6260	0.9139	0.8084	0.7099	1.3355	1.5198

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 µM	40 µM
10	1.6087	1.6130	1.4837	1.5928	1.4623	2.3656	2.3285	2.3073
15	1.5718	1.5223	1.3492	1.4603	1.3978	2.2380	2.2145	2.1863
20	1.5610	1.5007	1.1966	1.4159	1.3804	2.1663	2.1204	2.0901
25	1.5513	1.4939	1.1613	1.1770	1.1698	2.0834	2.0500	2.0211
30	1.5495	1.4904	0.9508	1.0366	0.9716	2.0319	2.0026	1.9753
35	1.5429	1.4793	0.9335	0.9563	0.7966	1.9937	1.9648	1.9368
40	1.5368	1.4755	0.7697	0.7974	0.7555	1.9662	1.9372	1.9061
45	1.5360	1.4066	0.7410	0.7101	0.7393	1.9387	1.9132	1.8878

Table B-107.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 20Apr2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	1.5585	1.5218	1.5997	2.1958	2.1520	2.0326	2.0790	2.1122	1.6893	1.6865
15	1.4630	1.2384	1.3899	1.9784	1.9556	2.0249	2.0759	2.0625	1.6719	1.6851
20	1.3959	1.2660	1.3658	1.8706	1.9537	1.8523	2.0666	1.7723	1.6245	1.6847
25	1.2987	1.2332	1.1859	1.7730	1.9057	1.5323	2.0631	1.5123	1.4026	1.6454
30	1.2991	1.3020	1.1484	1.6779	1.8541	1.4181	2.0683	1.4805	1.1663	1.5630
35	1.2428	1.2330	1.1225	1.7433	1.8013	1.3480	2.0618	1.5511	1.0607	1.3937
40	1.2489	1.1089	1.1508	1.6196	1.8264	1.2949	2.0176	1.4516	1.0658	1.3756
45	1.1287	1.0925	1.1322	1.5558	1.8036	1.2480	1.8508	1.3070	0.9834	1.2009

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 µM	40 µM
10	1.6966	1.6121	2.2530	2.2906	2.1095	2.0942	2.1490	2.2106
15	1.6815	1.5991	2.2553	2.1352	2.0823	2.0635	2.1092	2.1710
20	1.5468	1.4774	2.2664	2.0805	2.0748	2.0392	2.0907	2.1463
25	1.3719	1.1995	2.2674	1.8479	1.9508	2.0279	2.0718	2.1367
30	1.1215	1.0647	2.2653	1.8373	1.9080	2.0141	2.0724	2.1166
35	1.0698	1.0750	2.2586	1.6903	1.7725	2.0034	2.0583	2.1149
40	1.0831	0.9262	2.2506	1.7452	1.6385	1.9992	2.0550	2.1102
45	0.8942	0.8904	2.2303	1.7419	1.4518	1.9973	2.0524	2.1077

Table B-107.1c. Batch 1, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 20April2012

Time	Set B1					Set B2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B1-0	B1-10	B1-20	B1-30	B1-40	B2-0	B2-10	B2-20	B2-30	B2-40
10	2.2146	2.1745	2.0921	2.1955	1.9147	2.3328	2.1395	2.3311	2.3832	2.2336
15	2.2128	2.1541	2.0552	2.1483	1.8625	2.3276	2.1193	2.3047	2.3464	2.1969
20	2.2069	2.1391	2.0285	2.1148	1.8209	2.3219	2.1056	2.2857	2.3159	2.1566
25	2.1962	2.1257	2.0048	2.0875	1.7974	2.3111	2.0921	2.2706	2.2867	2.1186
30	2.1919	2.1187	1.9839	2.0618	1.7616	2.3092	2.0814	2.2580	2.2764	2.0905
35	2.1856	2.1068	1.9727	2.0459	1.7416	2.3083	2.0672	2.2490	2.2559	2.0687
40	2.1834	2.0942	1.9579	2.0294	1.7245	2.3018	2.0642	2.2397	2.2397	2.0450
45	2.1724	2.0935	1.9486	2.0132	1.7108	2.2846	2.0581	2.2283	2.2107	2.0236
50	2.1708	2.0856	1.9382	2.0023	1.6956	2.2859	2.0559	2.2198	2.2118	2.0134
55	2.1587	2.0798	1.9248	1.9917	1.6890	2.2933	2.0480	2.2103	2.2049	2.0055
60	2.1575	2.0749	1.9216	1.9829	1.6795	2.2786	2.0428	2.2090	2.1928	1.9948
Rate[a]	0.0012	0.0019	0.0032	0.0040	0.0045	0.0010	0.0018	0.0023	0.0037	0.0048
r^2	0.9867	0.9518	0.9411	0.9396	0.9279	0.9271	0.9364	0.9560	0.9476	0.9476
Rate – neg ctrl rate	0.0005	0.0012	0.0025	0.0033	0.0038	0.0003	0.0011	0.0016	0.0030	0.0042

Time	Set C1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C1-0	C1-10	C1-20	C1-30	C1-40	neg ctr	20 uM	40 uM
10	2.5426	2.4790	1.9490	2.1912	2.2147	2.3273	2.2147	2.2411
15	2.5119	2.4583	1.9052	2.1393	2.1480	2.3091	2.1567	2.1581
20	2.5307	2.4440	1.8799	2.1055	2.0967	2.3091	2.1228	2.1042
25	2.5284	2.4221	1.8540	2.0806	2.0579	2.3080	2.1010	2.0532
30	2.5043	2.4197	1.8309	2.0572	2.0255	2.3072	2.0798	2.0171
35	2.5160	2.4147	1.8170	2.0393	1.9995	2.3085	2.0601	1.9765
40	2.4963	2.4109	1.8051	2.0225	1.9730	2.3060	2.0381	1.9434
45	2.5060	2.3990	1.7945	2.0086	1.9551	2.2888	2.0197	1.9098
50	2.5002	2.3753	1.7852	1.9970	1.9418	2.2898	1.9920	1.8828
55	2.4963	2.3830	1.7780	1.9864	1.9223	2.2787	1.9824	1.8588
60	2.4855	2.3747	1.7710	1.9779	1.9135	2.2987	1.9729	1.8382
Rate[a]	0.00089	0.0020	0.0033	0.0040	0.0057	0.0007	0.0046	0.0077
r^2	0.7382	0.9401	0.9208	0.9343	0.9344	0.6885	0.9644	0.9664
Rate – neg ctrl rate	0.00019	0.0013	0.0026	0.0033	0.0050	0.0000	0.0039	0.0070

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-107.1c-B1, B2, C1, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
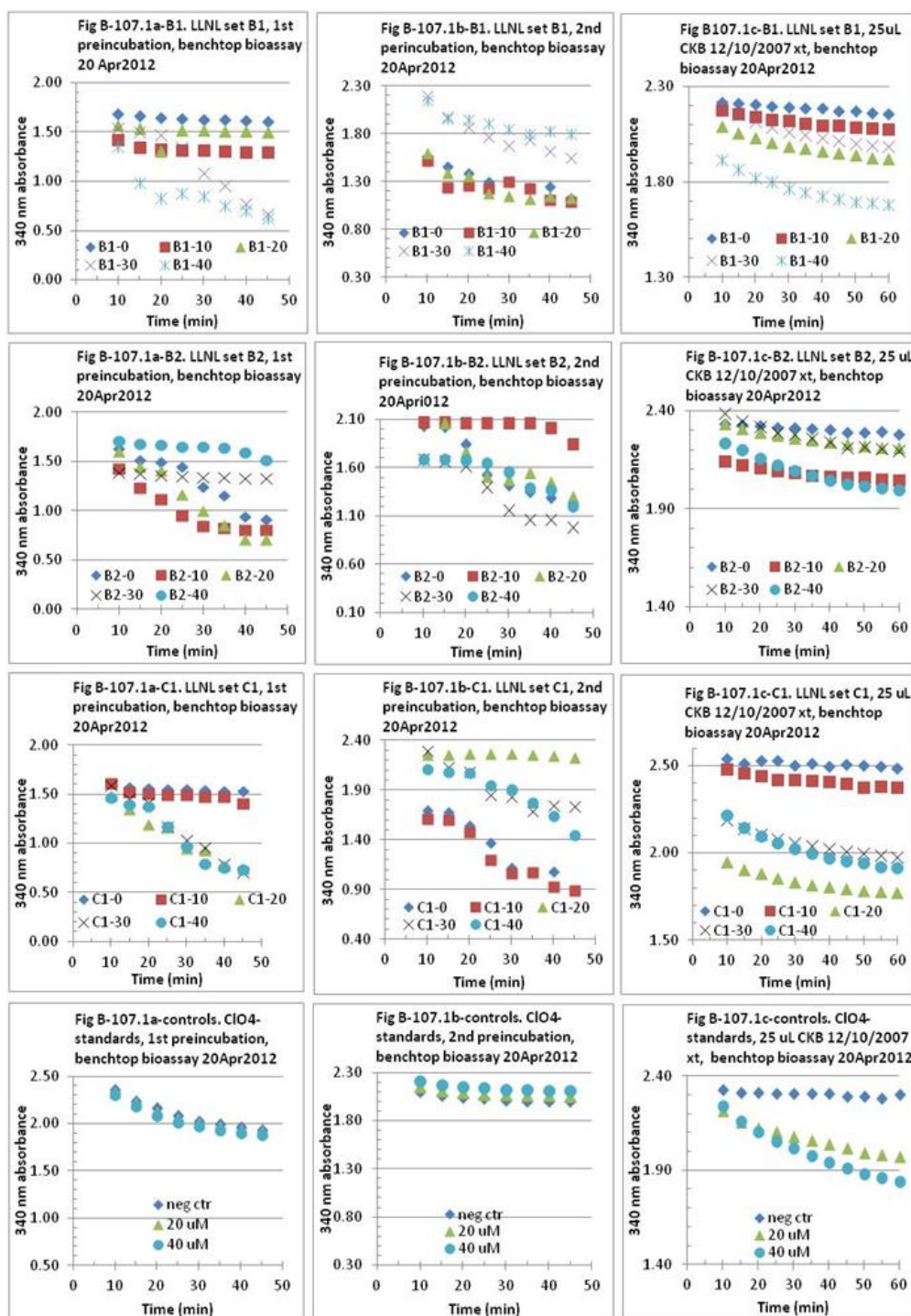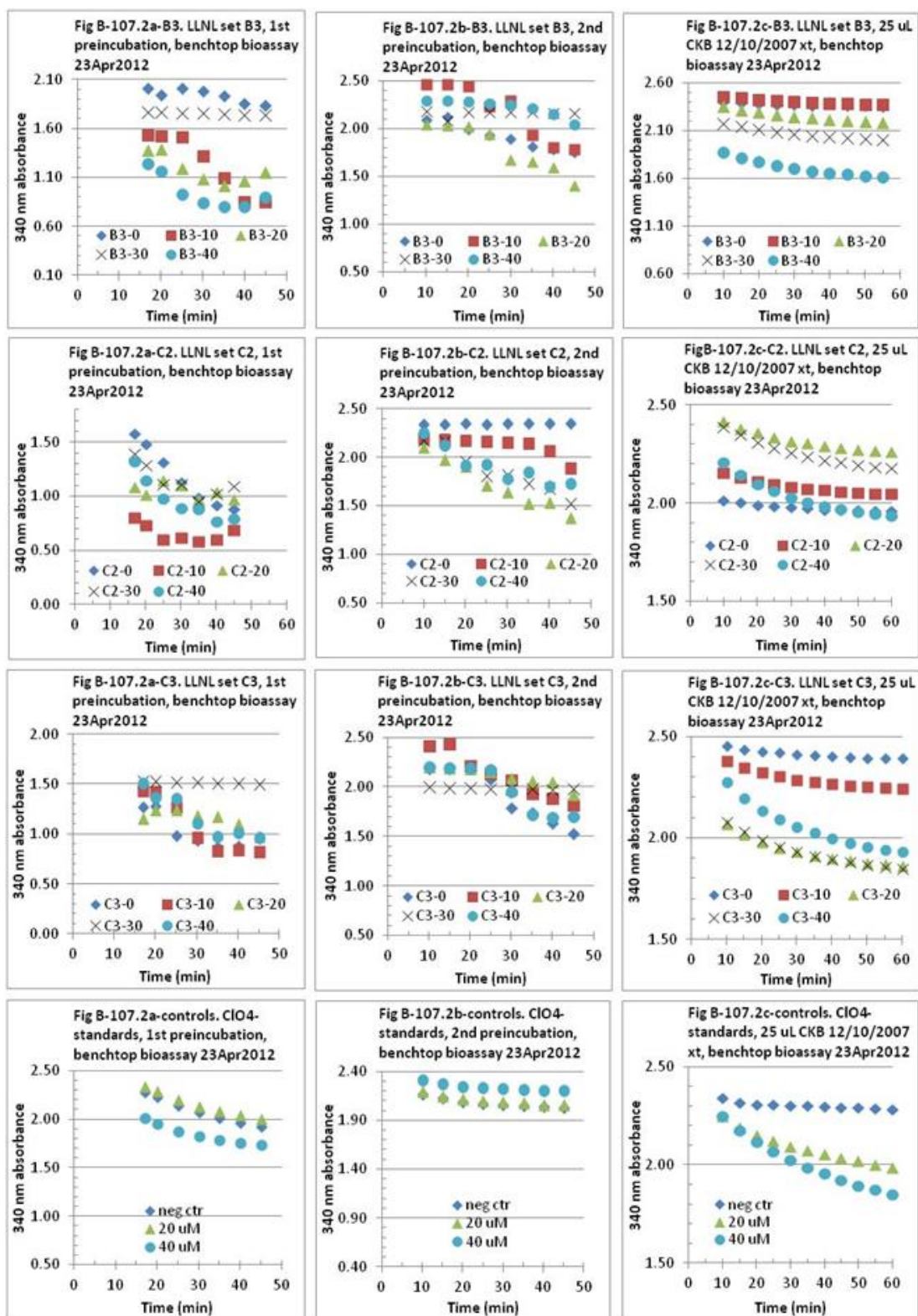
Figure B-107.2. Lawrence Livermore National Laboratory-site 300, batch 2, sets B3, C2, C3, and perchlorate controls. Test date 23 Apr 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-107.2a, b, and c

Data for Figure B-107.2.

Groundwater sample 107, Lawrence Livermore National Laboratory-site 300. Standards addition method benchtop bioassay, batch 2, 23April2012. SDVB/DTAB eluate sets B3, C2, C3, perchlorate 20 and 40 µM standards, and negative control (no ClO_4^- present)

Table B-107.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 23April2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
17	2.0098	1.5291	1.3783	1.7714	1.2431	1.5810	0.8077	1.0874	1.3944	1.3240
20	1.9438	1.5202	1.3843	1.7678	1.1597	1.4841	0.7385	1.0148	1.2945	1.1497
25	2.0065	1.5150	1.1924	1.7603	0.9255	1.3204	0.6064	1.1487	1.1162	0.9852
30	1.9780	1.3225	1.0821	1.7531	0.8380	1.1338	0.6226	1.1082	1.1175	0.8941
35	1.9359	1.0943	1.0142	1.7462	0.8023	0.9936	0.5829	0.9731	0.9560	0.8879
40	1.8531	0.8491	1.0646	1.7394	0.8030	0.9169	0.6044	1.0474	1.0271	0.7742
45	1.8364	0.8512	1.1491	1.7381	0.9037	0.8861	0.6881	0.9695	1.0925	0.7998

Time	Set C3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 µM	40 µM
17	1.2759	1.4336	1.1584	1.5349	1.5141	2.2807	2.3384	2.0127
20	1.2794	1.4255	1.2399	1.5313	1.3663	2.2305	2.2874	1.9568
25	0.9863	1.2740	1.2415	1.5251	1.3566	2.1414	2.1955	1.8792
30	0.9369	0.9638	1.1866	1.5189	1.1081	2.0744	2.1351	1.8268
35	0.8693	0.8351	1.1794	1.5135	0.9737	2.0159	2.0819	1.7890
40	0.8763	0.8448	1.0959	1.5099	1.0134	1.9692	2.0396	1.7554
45	0.9601	0.8270	0.9715	1.5058	0.9621	1.9290	2.0044	1.7337

Table B-107.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 23April2012

Time	Set B3					Set C2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	B3-0	B3-10	B3-20	B3-30	B3-40	C2-0	C2-10	C2-20	C2-30	C2-40
10	2.0996	2.4718	2.0445	2.1906	2.3000	2.3413	2.1909	2.1005	2.1753	2.2464
15	2.1257	2.4712	2.0404	2.0845	2.2971	2.3371	2.1819	1.9790	2.1609	2.1171
20	1.9941	2.4478	2.0284	2.1802	2.2917	2.3472	2.1702	1.9029	1.9552	1.9186
25	1.9412	2.2426	1.9446	2.1744	2.2681	2.3405	2.1643	1.7123	1.8058	1.9232
30	1.8998	2.2984	1.6743	2.1749	2.2539	2.3467	2.1548	1.6410	1.8138	1.7807
35	1.8189	1.9436	1.6619	2.1747	2.2143	2.3489	2.1456	1.5175	1.7277	1.8502
40	1.7932	1.8100	1.5995	2.1724	2.1565	2.3521	2.0665	1.5340	1.6824	1.7020
45	1.7554	1.7863	1.4055	2.1695	2.0484	2.3500	1.8847	1.3696	1.5249	1.7267

Time	Set C3					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	C3-0	C3-10	C3-20	C3-30	C3-40	neg ctr	20 µM	40 µM
10	2.1811	2.4118	2.2049	2.0052	2.2023	2.1649	2.1943	2.3108
15	2.1822	2.4366	2.1922	1.9958	2.1947	2.1251	2.1479	2.2735
20	2.1808	2.2138	2.1827	1.9866	2.1915	2.0890	2.1168	2.2432
25	2.0798	2.1397	2.1560	1.9846	2.1758	2.0704	2.0946	2.2305
30	1.7909	2.0685	2.0908	1.9802	1.9486	2.0531	2.0805	2.2193
35	1.7442	1.9298	2.0631	1.9786	1.7207	2.0434	2.0731	2.2103
40	1.6334	1.8832	2.0452	1.9757	1.6887	2.0359	2.0600	2.2051
45	1.5243	1.8166	1.9244	1.9781	1.7024	2.0311	2.0554	2.2065

Table B-107.2c. Batch 2, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 23April2012

Time	Set B3					Set C2				
	rxn 1	**rxn 2**	**Rxn 3**	**rxn 4**	**rxn 5**	**rxn6**	**rxn 7**	**rxn 8**	**rxn 9**	**rxn 10**
(min)	**B3-0**	**B3-10**	**B3-20**	**B3-30**	**B3-40**	**C2-0**	**C2-10**	**C2-20**	**C2-30**	**C2-40**
10	2.3994	2.4505	2.3508	2.1683	1.8736	2.0146	2.1563	2.4162	2.3911	2.2096
15	2.3896	2.4414	2.3081	2.1354	1.8122	2.0035	2.1318	2.3818	2.3483	2.1473
20	2.3797	2.4195	2.2843	2.1058	1.7651	1.9931	2.1123	2.3604	2.3098	2.0985
25	2.3731	2.4095	2.2601	2.0796	1.7261	1.9868	2.0970	2.3378	2.2812	2.0620
30	2.3648	2.3973	2.2387	2.0582	1.6947	1.9799	2.0825	2.3184	2.2562	2.0308
35	2.3626	2.3853	2.2244	2.0436	1.6684	1.9752	2.0751	2.3045	2.2378	2.0068
40	2.3910	2.3826	2.2090	2.0278	1.6487	1.9687	2.0672	2.2912	2.2201	1.9863
45	2.3596	2.3771	2.2000	2.0150	1.6341	1.9685	2.0595	2.2841	2.2096	1.9730
50	2.3554	2.3710	2.1879	2.0088	1.6194	1.9647	2.0551	2.2728	2.1957	1.9557
55	2.3533	2.3671	2.1825	2.0006	1.6088	1.9617	2.0511	2.2680	2.1868	1.9450
60	2.3481	2.3704	2.1762	1.9925	1.5969	1.9624	2.0481	2.2620	2.1809	1.9370
Rate[a]	0.00085	0.00186	0.00357	0.00366	0.00563	0.00102	0.00205	0.00295	0.00403	0.00513
r^2	0.6261	0.9421	0.9422	0.9464	0.9319	0.9151	0.9114	0.9349	0.9296	0.9197
Rate – neg ctrl rate	-0.00004	0.00097	0.00268	0.00277	0.00474	0.00013	0.00116	0.00206	0.00314	0.00424

Time	Set C3					Controls		
	rxn 11	**rxn 12**	**rxn 13**	**rxn 14**	**rxn 15**	**rxn 16**	**rxn 17**	**rxn 18**
(min)	**C3-0**	**C3-10**	**C3-20**	**C3-30**	**C3-40**	**neg ctr**	**20 µM**	**40 µM**
10	2.4554	2.3814	2.0674	2.0801	2.2765	2.3435	2.2524	2.2501
15	2.4389	2.3481	2.0157	2.0312	2.1982	2.3199	2.1931	2.1760
20	2.4266	2.3227	1.9798	1.9913	2.1366	2.3065	2.1511	2.1176
25	2.4205	2.3052	1.9529	1.9584	2.0946	2.3069	2.1223	2.0704
30	2.4139	2.2884	1.9315	1.9309	2.0553	2.3037	2.0964	2.0270
35	2.4078	2.2785	1.9145	1.9118	2.0259	2.3050	2.0736	1.9901
40	2.4048	2.2667	1.9006	1.8959	2.0004	2.2963	2.0535	1.9570
45	2.4001	2.2591	1.8888	1.8792	1.9745	2.2935	2.0344	1.9262
50	2.3929	2.2533	1.8784	1.8669	1.9567	2.2913	2.0203	1.8978
55	2.3966	2.2485	1.8711	1.8593	1.9448	2.2895	2.0030	1.8753
60	2.3944	2.2417	1.8617	1.8495	1.9341	2.2860	1.9890	1.8524
Rate[a]	0.00114	0.00258	0.00376	0.00438	0.00647	0.00089	0.00489	0.00765
r^2	0.8921	0.9124	0.9117	.09272	0.9255	0.9067	0.9504	0.9677
Rate – neg ctrl rate	0.00025	0.00169	0.00287	0.00349	0.00558	0.00000	0.00400	0.00676

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-107.2c-B3, C2, C3, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture
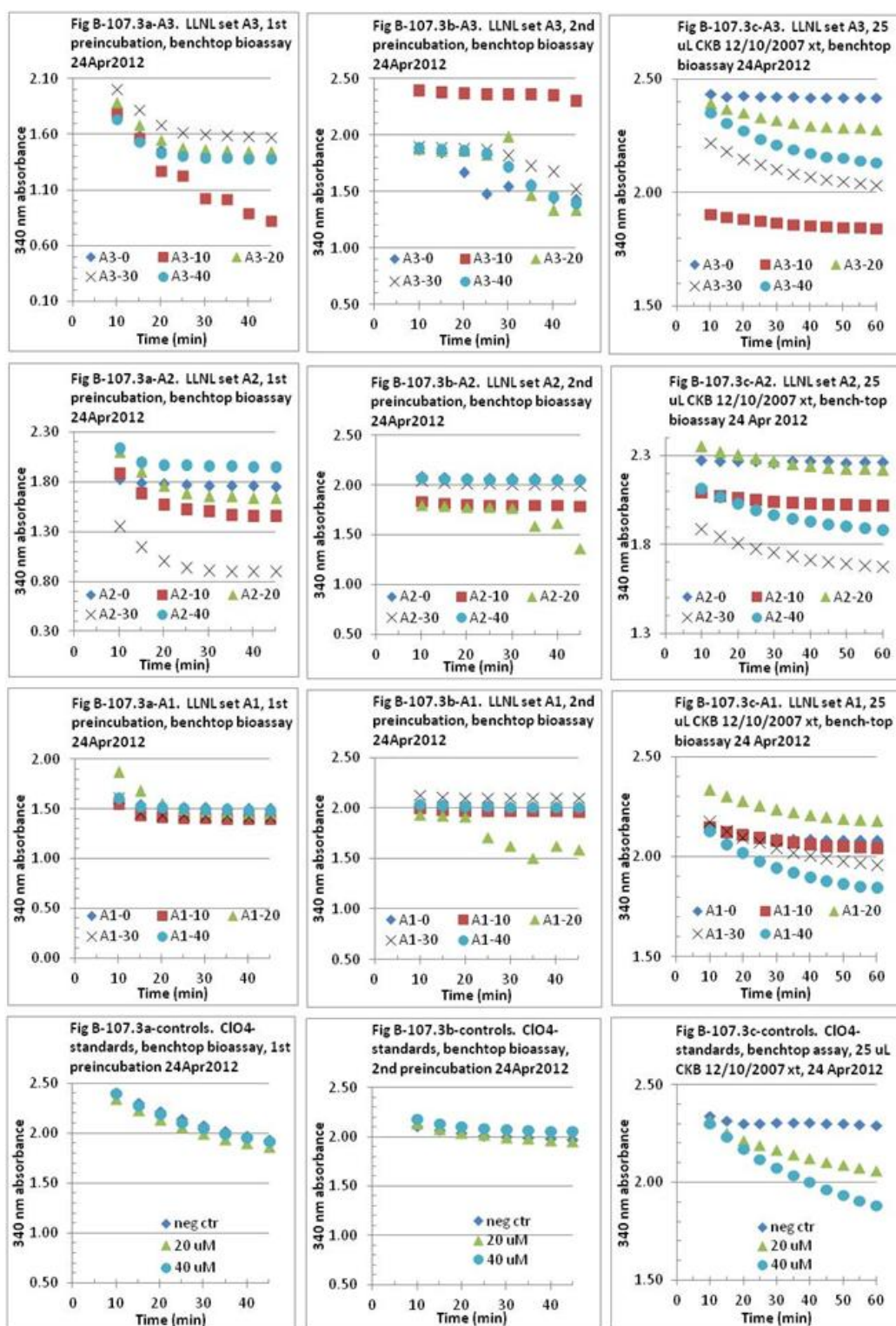
Figure B-107.3. Lawrence Livermore National Laboratory-site 300, batch 3, sets A3, A2, A1, and perchlorate controls. Test date 24 Apr 2012. 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 μL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-107.3a, b, and c

Data for Figure B-107.3.
Groundwater sample 107, Lawrence Livermore National Laboratory-site 300. Standards addition method benchtop bioassay, batch 3, 24April2012. SDVB/DTAB eluate sets A3, A2, A1, perchlorate 20 and 40 µM standards, and negative control (no ClO$_4^-$ present)

Table B-107.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 24April2012

Time	Set A3					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	1.6840	1.4191	1.5650	1.5357	1.3548	1.6318	1.4263	1.6015	1.3934	1.7087
15	1.6609	1.3451	1.5426	1.5003	0.9903	1.5195	1.2314	1.4463	1.3769	1.6782
20	1.6446	1.3291	1.3050	1.4750	0.8296	1.4943	1.1146	1.4132	1.3621	1.6690
25	1.6346	1.3201	1.5250	1.3479	0.8789	1.4455	0.9506	1.1711	1.3541	1.6544
30	1.6277	1.3135	1.5202	1.0839	0.8529	1.2461	0.8509	1.0072	1.3455	1.6546
35	1.6222	1.3046	1.5114	0.9625	0.7545	1.1538	0.8261	0.8586	1.3390	1.6397
40	1.6179	1.2999	1.5073	0.7694	0.7026	0.9419	0.8056	0.7105	1.3370	1.5929
45	1.6108	1.2952	1.5025	0.6672	0.6260	0.9139	0.8084	0.7099	1.3355	1.5198

Time	Set A1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctr	20 µM	40 µM
10	1.6087	1.6130	1.4837	1.5928	1.4623	2.3656	2.3285	2.3073
15	1.5718	1.5223	1.3492	1.4603	1.3978	2.2380	2.2145	2.1863
20	1.5610	1.5007	1.1966	1.4159	1.3804	2.1663	2.1204	2.0901
25	1.5513	1.4939	1.1613	1.1770	1.1698	2.0834	2.0500	2.0211
30	1.5495	1.4904	0.9508	1.0366	0.9716	2.0319	2.0026	1.9753
35	1.5429	1.4793	0.9335	0.9563	0.7966	1.9937	1.9648	1.9368
40	1.5368	1.4755	0.7697	0.7974	0.7555	1.9662	1.9372	1.9061
45	1.5360	1.4066	0.7410	0.7101	0.7393	1.9387	1.9132	1.8878

Table B-107.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 24Apr2012

Time	Set A3					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	1.5585	1.5218	1.5997	2.1958	2.1520	2.0326	2.0790	2.1122	1.6893	1.6865
15	1.4630	1.2384	1.3899	1.9784	1.9556	2.0249	2.0759	2.0625	1.6719	1.6851
20	1.3959	1.2660	1.3658	1.8706	1.9537	1.8523	2.0666	1.7723	1.6245	1.6847
25	1.2987	1.2332	1.1859	1.7730	1.9057	1.5323	2.0631	1.5123	1.4026	1.6454
30	1.2991	1.3020	1.1484	1.6779	1.8541	1.4181	2.0683	1.4805	1.1663	1.5630
35	1.2428	1.2330	1.1225	1.7433	1.8013	1.3480	2.0618	1.5511	1.0607	1.3937
40	1.2489	1.1089	1.1508	1.6196	1.8264	1.2949	2.0176	1.4516	1.0658	1.3756
45	1.1287	1.0925	1.1322	1.5558	1.8036	1.2480	1.8508	1.3070	0.9834	1.2009

Time	Set A1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctr	20 μM	40 μM
10	1.6966	1.6121	2.2530	2.2906	2.1095	2.0942	2.1490	2.2106
15	1.6815	1.5991	2.2553	2.1352	2.0823	2.0635	2.1092	2.1710
20	1.5468	1.4774	2.2664	2.0805	2.0748	2.0392	2.0907	2.1463
25	1.3719	1.1995	2.2674	1.8479	1.9508	2.0279	2.0718	2.1367
30	1.1215	1.0647	2.2653	1.8373	1.9080	2.0141	2.0724	2.1166
35	1.0698	1.0750	2.2586	1.6903	1.7725	2.0034	2.0583	2.1149
40	1.0831	0.9262	2.2506	1.7452	1.6385	1.9992	2.0550	2.1102
45	0.8942	0.8904	2.2303	1.7419	1.4518	1.9973	2.0524	2.1077

Table B-107.3c. Batch 3, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 24April2012

Time	Set A3					Set A2				
	rxn 1	rxn 2	rxn 3	rxn 4	rxn 5	rxn 6	rxn 7	rxn 8	rxn 9	rxn 10
(min)	A3-0	A3-10	A3-20	A3-30	A3-40	A2-0	A2-10	A2-20	A2-30	A2-40
10	2.3994	2.4505	2.3508	2.1683	1.8736	2.0146	2.1563	2.4162	2.3911	2.2096
15	2.3896	2.4414	2.3081	2.1354	1.8122	2.0035	2.1318	2.3818	2.3483	2.1473
20	2.3797	2.4195	2.2843	2.1058	1.7651	1.9931	2.1123	2.3604	2.3098	2.0985
25	2.3731	2.4095	2.2601	2.0796	1.7261	1.9868	2.0970	2.3378	2.2812	2.0620
30	2.3648	2.3973	2.2387	2.0582	1.6947	1.9799	2.0825	2.3184	2.2562	2.0308
35	2.3626	2.3853	2.2244	2.0436	1.6684	1.9752	2.0751	2.3045	2.2378	2.0068
40	2.3910	2.3826	2.2090	2.0278	1.6487	1.9687	2.0672	2.2912	2.2201	1.9863
45	2.3596	2.3771	2.2000	2.0150	1.6341	1.9685	2.0595	2.2841	2.2096	1.9730
50	2.3554	2.3710	2.1879	2.0088	1.6194	1.9647	2.0551	2.2728	2.1957	1.9557
55	2.3533	2.3671	2.1825	2.0006	1.6088	1.9617	2.0511	2.2680	2.1868	1.9450
60	2.3481	2.3704	2.1762	1.9925	1.5969	1.9624	2.0481	2.2620	2.1809	1.9370
Rate[a]	0.00027	0.00121	0.00222	0.00359	0.00420	0.00021	0.00139	0.00252	0.00412	0.00440
r^2	0.7706	0.9441	0.9124	0.9357	0.9259	0.4909	0.9007	0.9187	0.9370	0.9288
Rate – neg ctrl rate	-0.00034	0.00060	0.00161	0.00298	0.00359	-0.00040	0.00078	0.00191	0.00351	0.00379

Time	Set A1					Controls		
	rxn 11	rxn 12	rxn 13	rxn 14	rxn 15	rxn 16	rxn 17	rxn 18
(min)	A1-0	A1-10	A1-20	A1-30	A1-40	neg ctr	20 µM	40 µM
10	2.4554	2.3814	2.0674	2.0801	2.2765	2.3435	2.2524	2.2501
15	2.4389	2.3481	2.0157	2.0312	2.1982	2.3199	2.1931	2.1760
20	2.4266	2.3227	1.9798	1.9913	2.1366	2.3065	2.1511	2.1176
25	2.4205	2.3052	1.9529	1.9584	2.0946	2.3069	2.1223	2.0704
30	2.4139	2.2884	1.9315	1.9309	2.0553	2.3037	2.0964	2.0270
35	2.4078	2.2785	1.9145	1.9118	2.0259	2.3050	2.0736	1.9901
40	2.4048	2.2667	1.9006	1.8959	2.0004	2.2963	2.0535	1.9570
45	2.4001	2.2591	1.8888	1.8792	1.9745	2.2935	2.0344	1.9262
50	2.3929	2.2533	1.8784	1.8669	1.9567	2.2913	2.0203	1.8978
55	2.3966	2.2485	1.8711	1.8593	1.9448	2.2895	2.0030	1.8753
60	2.3944	2.2417	1.8617	1.8495	1.9341	2.2860	1.9890	1.8524
Rate[a]	0.00085	0.00199	0.00304	0.00410	0.00537	0.00061	0.00471	0.00817
r^2	0.8301	0.9211	0.9330	0.9408	0.9264	0.6284	0.9495	0.9750
Rate – neg ctrl rate	0.00024	0.00138	0.00243	0.00349	0.00476	0.00000	0.00410	0.00756

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-107.3c-A3, A2, A1, and controls. r^2 is the correlation coefficient for the corresponding regression line for each reaction mixture

Appendix C: PERCHLORATE IN GROUNDWATER SAMPLES TESTED BY REFERENCE METHODS IN COMMERCIAL LABORATORIES

Table C-1. Perchlorate (μg/L) determined by reference analytical methods in commercial laboratories[a]

Site	102, Hill AFB	103, NWIRP McGregor	104, Jet Propulsion Laboratory	105, Fontana Water Co.	106, Massachusetts Military Reservation	107, Lawrence Livermore National Laboratory
Location	U9-16-007	OFFWS-37	MW-16	F-17C	J3-INF	W-854-1823
Collection date	14Jul2011	13Oct2011	14Nov2011	25Jan2012	23Feb2012	29Mar2012
Analysis date	22Jul2011	19Oct2011	21Nov2011	09Feb2012	06Mar2012	12Apr2012
Instrumentation	IC	IC	IC	LC/MS	LC/MS	LC/MS
EPA method	314.0	314.0	314.0	6850	6850	6850
Bottle A						
Replicate 1	36	59	ND	11	5.5	14
Replicate 2	40	60	ND	11	5.3	14
Replicate 3	32	61	ND	11	4.9	13
Bottle B						
Replicate 1	47	98	ND	11	5.2	15
Replicate 2	44	56	ND	11	5.3	14
Replicate 3	37	55	ND	11	5.3	13
Bottle C						
Replicate 1	33	NA	ND	9.3	4.9	12
Replicate 2	35	NA	ND	9.8	4.9	13
Replicate 3	38	NA	ND	9.5	4.8	12
Average ± SD	38±5	65±16	<2	11±1	5.1±0.3	13±1

[a]NA designates not applicable and ND designates none detected, *i.e.* less than the 2 μg/L reporting limit. The average is listed ± standard deviation

Appendix D: PERCHLORATE IN GROUNDWATER SAMPLES TESTED BY EPA METHOD 314.0 AT UNIVERSITY OF CALIFORNIA, BERKELEY

Table D-1. Perchlorate (μg/L) determined by EPA Method 314.0 at University of California, Berkeley[a]

Site	102, Hill AFB	103, NWIRP McGregor	104, Jet Propulsion Laboratory	105, Fontana Water Co.	106, Massachusetts Military Reservation	107, Lawrence Livermore National Laboratory
Location	U9-16-007	OFFWS-37	MW-16	F-17C	J3-INF	W-854-1823
Collection date	14Jul2011	13Oct2011	14Nov2011	25Jan2012	23Feb2012	29Mar2012
Analysis date	11Aug2011	09Nov2011	14Dec2011	24Feb2012	22Mar2012	25Apr2012
Instrumentation	IC	IC	IC	IC	IC	IC
EPA method	314.0	314.0	314.0	314.0	314.0	314.0
Bottle A						
Replicate 1	34	58	ND	11	6	16
Replicate 2	35	58	ND	11	6	16
Replicate 3	35	59	ND	11	6	16
Bottle B						
Replicate 1	35	54	ND	11	6	15
Replicate 2	35	55	ND	11	7	15
Replicate 3	36	55	ND	11	6	15
Bottle C						
Replicate 1	36	NA	ND	11	6	16
Replicate 2	36	NA	ND	10	6	16
Replicate 3	36	NA	ND	10	6	16
Average ± SD	35±1	57±2	<4	11±0	6±0	16±1

[a]NA designates not applicable and ND designates none detected, *i.e.* less than the 4 μg/L reporting limit. The average is listed ± standard deviation

Appendix E: CALIBRATION PROCEDURES, QUALITY ASSURANCE SAMPLING, DECONTAMINATION PROCEDURES, AND SAMPLE DOCUMENTATION

E-1.0   CALIBRATION OF ANALYTICAL EQUIPMENT

Ion chromatographs and analytical balances receive anual preventive maintenance inspection and calibration by the manufacturer or the manufacturer's designate. In addition, calibration standards are analyzed with each batch of samples analyzed by ion chromatography.

Conductivity standards are analyzed before use as specified in the operator's manual.

Standards for the pH meter are used daily to calibrate the pH meter, as specified in the operator's manual.

The alignment of the UV/Vis spectrophotometer is checked before use.

E-2.0   QUALITY ASSURANCE SAMPLING

Three one gallon amounts of groundwater were collected successively in different bottles for each sampling event. This provided field replicates for testing.

The contents of each bottle were subsequently tested in triplicate by each of the three perchlorate analysis methods (benchtop bioassay, IC or LC/MS performed by a commercial laboratory, and IC performed in the laboratory of the PI)

E-3.0   DECONTAMINATION PROCDURES

Decontamination procedures are not applicable to the project.

E-4.0   SAMPLE DOCUMENTATION

E-4.1   SHIPPING AND COLLECTION OF GROUNDWATER SAMPLES

Bottles were prelabeled and shipped in a sealed cooler with a chain-of-custody form to the site. The samples were collected by site personnel who shipped them to the PI's laboratory for next day delivery in the sealed cooler with the chain-of-custody form.

In addition to documenting receipt of the shipment on the chain-of-custody form, receipt was also documented in the bound laboratory notebook dedicated to the project.

E-4.2   DOCUMENTATION FOR SUBSAMPLES ANALYZED BY COMMERCIAL LABORATORIES

Triplicate subsamples were prepared from each gallon of groundwater in labeled bottles. These were shipped for next day delivery in a sealed cooler with a chain-of-custody form to the commercial laboratory.

The operations undertaken to prepare these subsamples were documented in the bound laboratory notebook dedicated to the project.

E-4.3   DOCUMENTATION FOR SUBSAMPLES ANALYZED IN THE PI'S LABORATORY

Triplicate subsamples for bioassay analysis and IC analysis in the PI's laboratory were prepared in tubes or bottles and project-specific labels identified the contents with regard to groundwater source and the dates of source collection and subsample preparation.

E-4.4   DOCUMENTATION FOR SPE ELUATE PREPARATION AND BENCHTOP BIOASSAY TESTING

Steps in the preparation of SPE eluates and the dates operations were performed were documented on project specific test records.

Absorbance readings at 340 nm for perchlorate reductase reactions in cuvettes were measured at timed intervals. These data were recorded on test records and constitute the primary data for benchtop bioassay testing.

Appendix F: MICROPLATE READER BIOASSAY DATA

In addition to benchtop bioassay testing (see Appendix B), 3 to 5 SPE eluate sets from the different groundwater sources were tested by the plate reader format of the bioassay, which is performed in an anerobic chamber using a 96-well microplate reader. Results are presented in Table F-1. There were five testing batches, identified with Roman numerals in Table F-1.

Table F-1. Perchlorate determined by plate reader bioassay for some SPE eluate sets with storage for various periods at 2 – 8 °C prior to analysis[a]

Site	Para-meter[b]	A1	A2		A3	B1	B2	B3	C1	C2	C3	Average ± SD[c]
102, Hill AFB	SPE date	27 Jul 2011	27 Jul 2011		27 Jul 2011	6 Aug 2011	-	-	-	-	9 Aug 2011	34±10 (batch I)
	PR batch	I	I	V	I	V	-	-	-	-	V	
	µg/L	36±4	43±14	27±5	23±4	38±17	-	-	-	-	34±4	33±6 (batch V)
103, NWIRP McGregor	SPE date	-	5 Nov 2011		5 Nov 2011	-	8 Nov 2011	-	NA	NA	NA	
	PR batch	-	III		II	-	IV	-				
	µg/L	-	53±6		45±12	-	49±4	-				49±4
104, JPL	SPE date	-	8 Dec 2011		-	-	-	11 Dec 2011	-	11 Dec 2011	-	
	PR batch	-	II		-	-	-	III	-	IV	-	
	µg/L	-	-10±7		-	-	-	2±2	-	-3±3	-	-4±6
105, Fontana Water Co.	SPE date	13 Feb 2012	13 Feb 2012		-	-	-	15 Feb 2012	-	-	17 Feb 2012	
	PR batch	II	III		-	-	-	IV	-	-	V	
	µg/L	19±5	11±1		-	-	-	8±2	-	-	10±1	12±5
106, MMR	SPE date	-	12 Mar 2012		12 Mar 2012	13 Mar 2012	-	-	-	14 Mar 2012	-	
	PR batch	-	V		II	III	-	-	-	IV	-	
	µg/L	-	3±1		4±3	8±2	-	-	-	4±0.4	-	5±2
107, LLNL	SPE date	-	-		-	12 Apr 2012	16 Apr 2012	-	17 Apr 2012	-	-	
	PR batch	-	-		-	II	III	-	IV	-	-	
	µg/L	-	-		-	13±3	14±3	-	6±2	-	-	11±4

[a]NA denotes not applicable, "-" denotes that there is no data for this cell of the table

[b]SPE date corresponds to the preparation date for an SPE eluate set. The plate reader bioassay batches are identified as I (tested on 5 Aug 2011), II (tested on 29 Jun 2012), III (tested on 6 Jul 2012), and IV and V (tested successively on 11 Jul 2012)

[c]The spread around the average is given as the standard deviation

The bioassay rates (i.e. 340 nm absorbance change/minute) used in the standard additions method to determine the perchlorate (µg/L) for an SPE eluate set are listed for the different

batches in Tables F-2 (batch I), F-3 (batch II), F-4 (batch III), F-5 (batch IV), and F-6 (batch V). Each is accompanied by a figure showing the standard additions method regression lines for the SPE eluate sets in the batch, from which the perchlorate concentration is determined. Additionally, several subsequent figures for each batch present the experimental data, i.e. the 340 nm absorbance over time for eluates. The perchlorate spikes added were 0, 10, 20, 30, or 40 µg/L.

Table F-2. Perchlorate estimated in plate reader bioassay batch 1 by the standard additions method for Hill SPE eluate sets A1, A2, A3, tested 5 Aug 2011[a]

Perchlorate Spike (µg/L)	102, Set A1	102, Set A2	102, Set A3	Perchlorate Standards	
				µM	Rate
0	0.001153	0.001118	0.000884	0	0.000000
10	0.001532	0.002108	0.001328	10	0.000640
20	0.001877	0.002169	0.001389	20	0.001160
30	0.002006	0.002247	0.001917	30	0.001655
40	0.002535	0.002689	0.002445	40	0.002283
Regression parameters for each set[b]					
y-intercept ± error	0.0011728 ±0.000075	0.0014100 ±0.00022	0.0008503 ±0.00012		0.0000314 ±0.000036
Slope ± error	0.0000324 ±0.0000031	0.0000328 ±0.0000092	0.0000371 ±0.0000048		0.0000558 ±0.000001
r^2	0.9736	0.8080	0.9529		0.9979
x-intercept ± error	-36±4.2	-43±14	-23±4.3		-0.56±0.65
ClO_4^- in groundwater ± error, (µg/L)[c]	36±4	43±14	23±4		NA

[a]The eluate sets were tested in duplicate and the values listed are the average rate (340 nm AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. The negative control rate was 0.000501 AU/minute. Perchlorate reductase was added to microplate wells in 10 µL of CKB 8/3/2011 extract. NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure F-1). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((\text{slope error/slope})^2 + (\text{y-intercept error/y-intercept})^2)}$
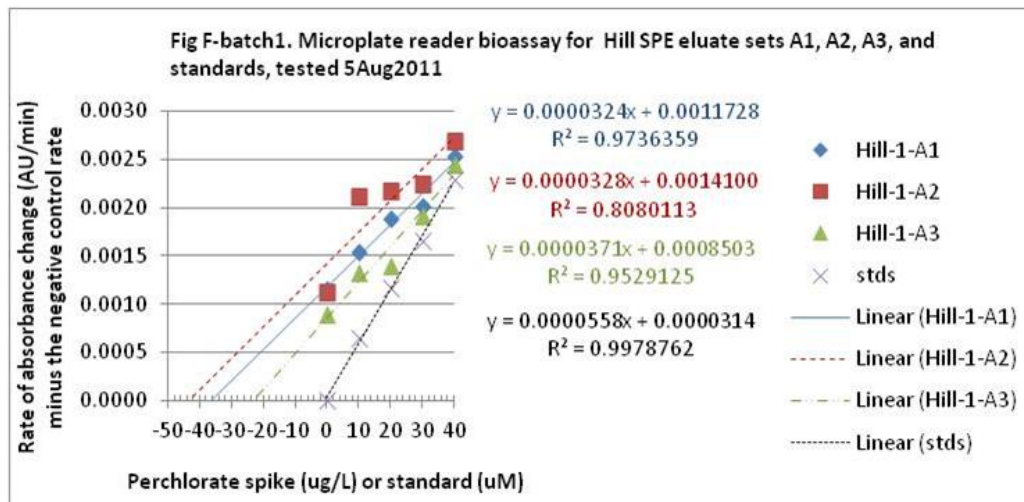

Figure F-1. Perchlorate by the standard additions method in plate reader bioassay batch 1 for Hill SPE eluate sets A1, A2, A3, and control standards, tested 5 Aug 2011
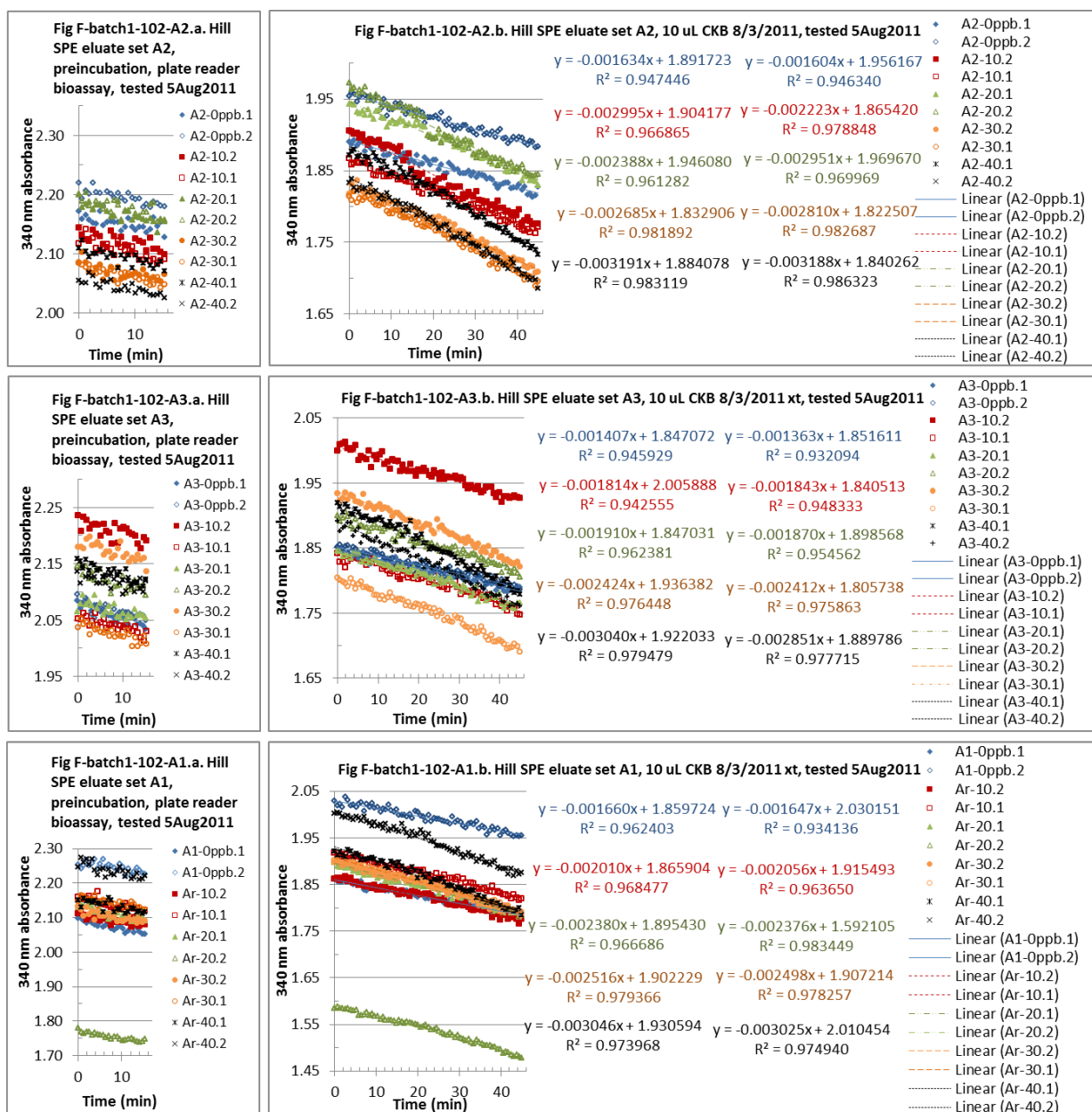
Figure F-1i. Plate reader bioassay batch 1 absorbance change for SPE eluate sets Hill A1, A2, and A3, 10 µL CKB 8/3/2011 extract, tested 5 Aug 2011
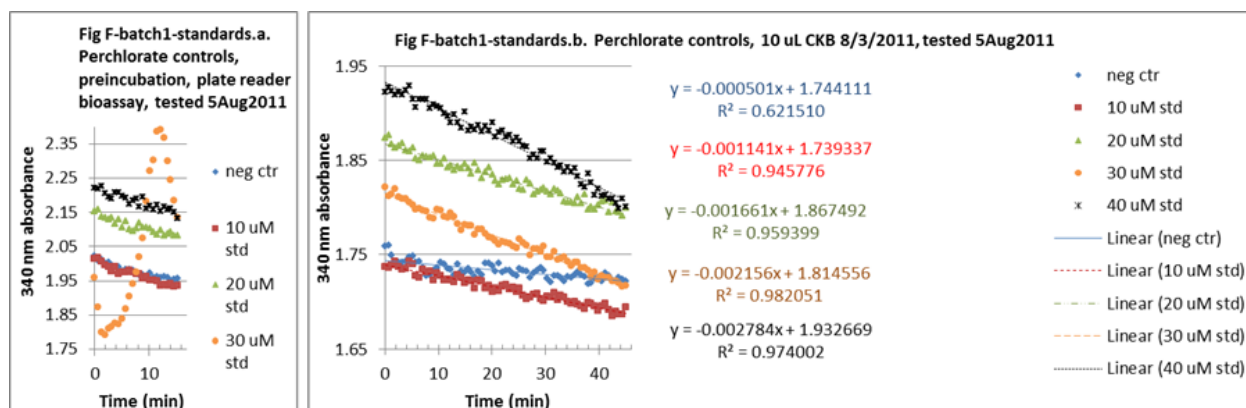
Figure F-1ii. Plate reader bioassay batch 1 absorbance change for control standards, 10 µL CKB 8/3/2011 extract, tested 5 Aug 2011

Table F-3. Perchlorate estimated in plate reader bioassay batch 2 by the standard additions method for SPE eluate sets FWC-A1, JPL-B1, LLNL-B1, McGregor-A3, and MMR-A3, tested 29 Jun 2012[a]

Perchlorate Spike (µg/L)	105, Fontana Water Co-A1	104, JPL-B1	107, LLNL-B1	103, NWIRP McGregor–A3	106, MMR-A3
0	0.001286	-0.001376	0.000527	0.003735	0.000526
10	0.001739	0.000011	0.001468	0.003979	0.000827
20	0.002006	0.001501	0.001854	0.004189	0.001904
30	0.002631	0.002788	0.002515	0.006197	0.003207
40	0.003762	0.002039	0.002765	0.006419	0.003563
Regression parameters for each set[b]					
y-intercept ± error	0.001116 ±0.00023	-0.000929 ±0.00062	0.000721 ±0.00016	0.003387 ±0.00043	0.000315 ±0.00025
Slope ± error	0.000058 ±0.0000093	0.000096 ±0.000025	0.000055 ±0.0000064	0.000076 ±0.000018	0.000085 ±0.000010
r^2	0.9293	0.8277	0.9614	0.8589	0.9584
x-intercept ± error	-19±4.9	9.7±6.9	-13±3.2	-45±12	-3.7±3.0
ClO_4^- in groundwater ± error, (µg/L)[c]	19±5	-10±7	13±3	45±12	4±3

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate (n = 5) was 0.000466 AU/minute. Perchlorate reductase was added to microplate wells in 10 µL of CKB 5/8/2012 extract

[b]Regression parameters are from the regression line for each set (see Figure F-2). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike)

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((\text{slope error/slope})^2 + (\text{y-int error/y-int})^2)}$
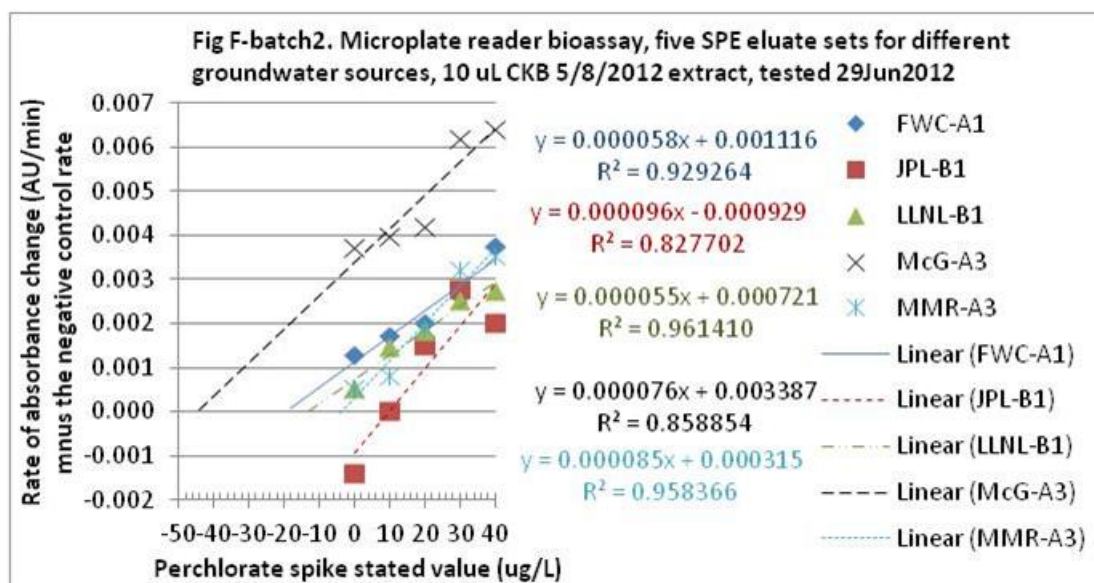

Figure F-2. Perchlorate estimated in plate reader bioassay batch 2 by the standard additions method for SPE eluate sets FWC A1, JPL B1, LLNL B1, NWIRP McGregor A3, and MMR A3, tested 29 Jun 2012
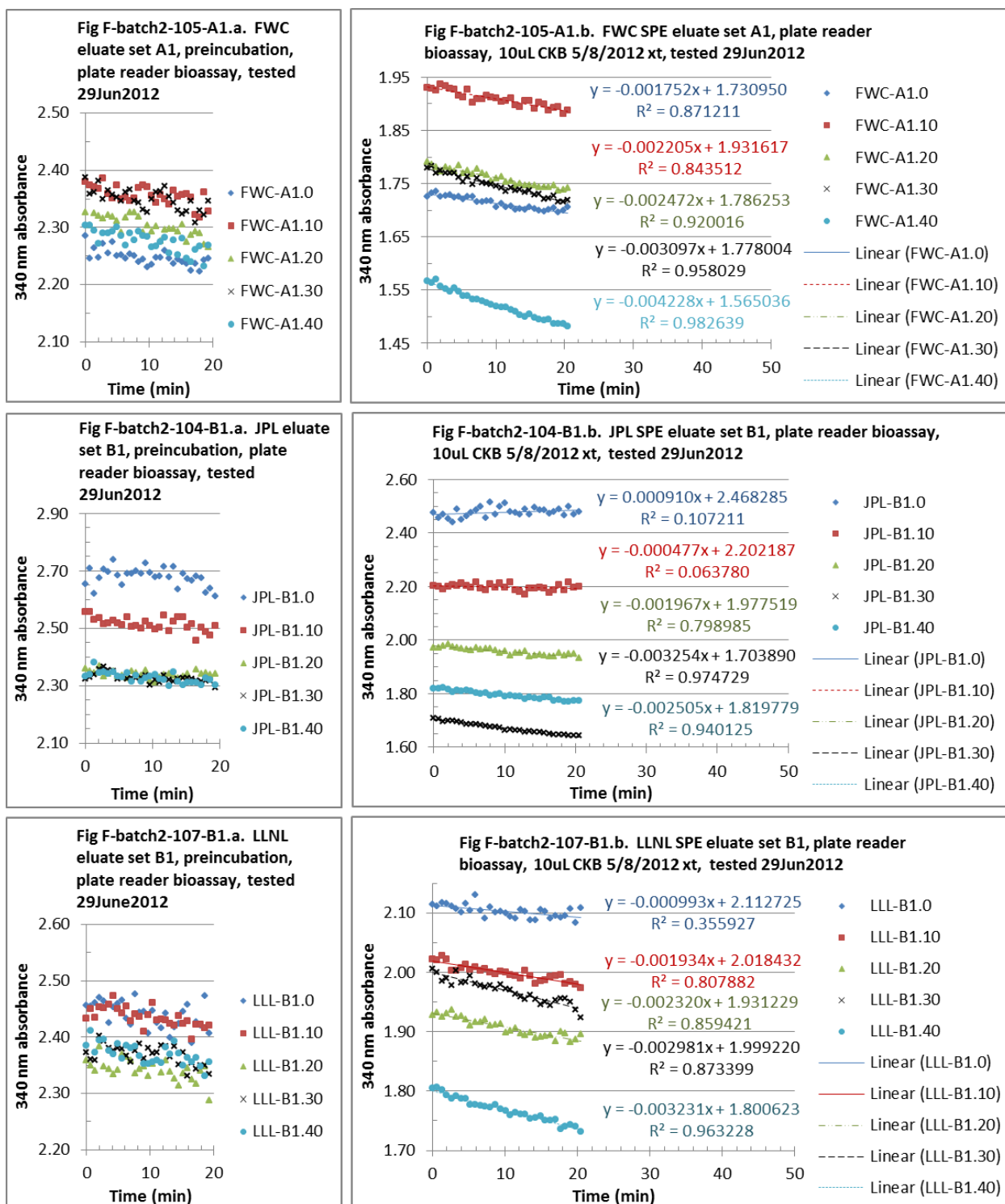
Figure F-2i. Plate reader bioassay batch 2 absorbance change for SPE eluate sets FWC A1, JPL B1, and LLNL B1, 10 μL CKB 5/8/2012 extract, tested 29 Jun 2012
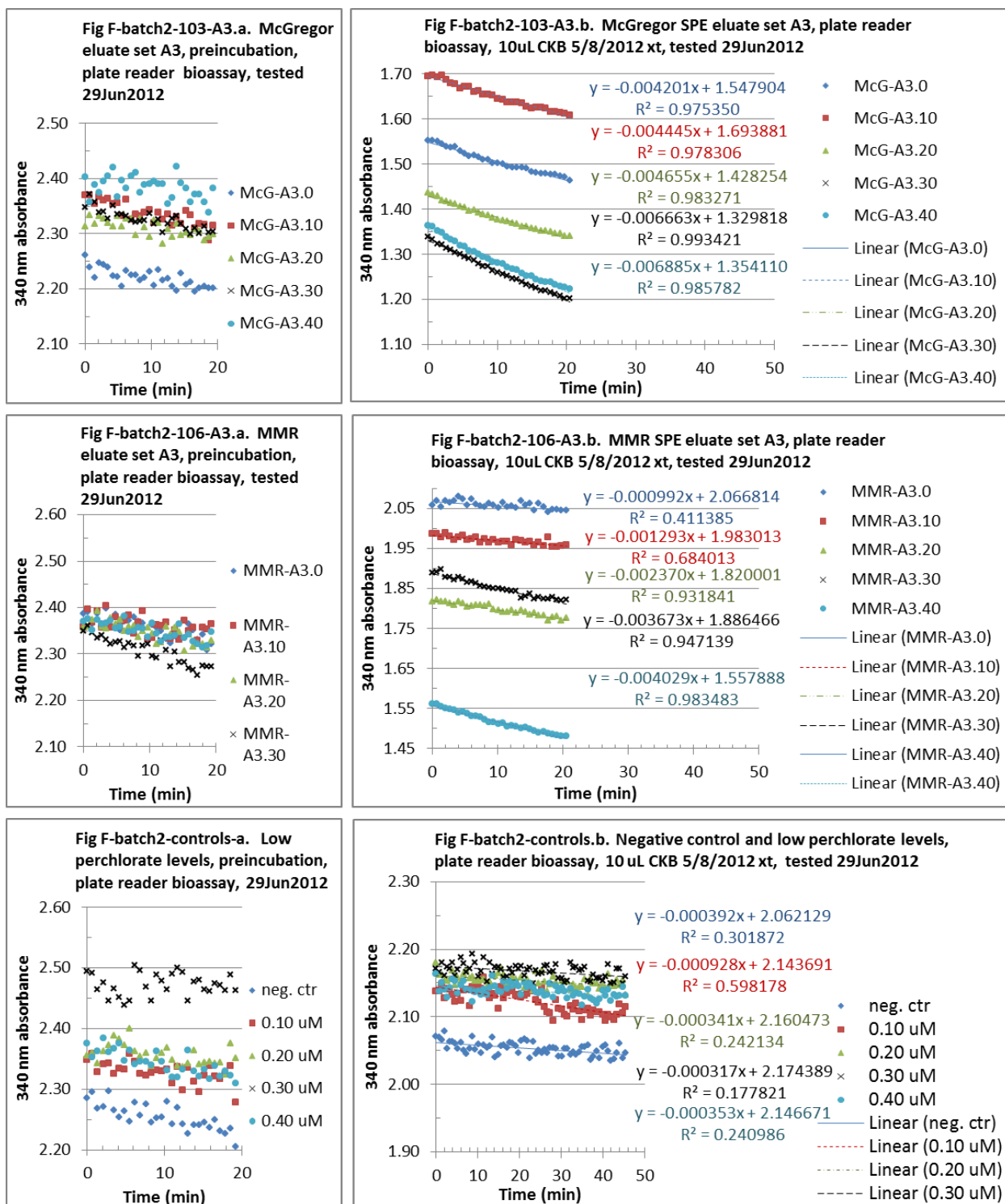
Figure F-2ii. Plate reader bioassay batch 2 absorbance change for SPE eluate sets McGregor A3, MMR A3, and negative control and low perchlorate levels of standards, 10 µL CKB 5/8/2012 extract, tested 29 Jun 2012

Table F-4. Perchlorate estimated in plate reader bioassay batch 3 by the standard additions method for SPE eluate sets FWC-A2, JPL-B3, LLNL-B2, NWIRP McGregor-A2, and MMR-B1, tested 6 Jul 2012[a]

Perchlorate Spike (µg/L)	105, FWC-A2	104, JPL-B3	107, LLNL-B2	103, NWIRP McGregor-A2	106, MMR-B1
0	0.0025738	0.0004478	0.0024498	0.0101648	0.0021588
10	0.0047358	0.0026238	0.0039738	0.0117088	0.0033608
20	0.0071228	0.0037818	0.0047168	0.0136018	0.0064428
30	0.0090988	0.0072858	0.0069978	0.0147608	0.0081748
40	0.0118418	0.0084258	0.0087638	0.0179888	0.0107048
Regression parameters for each set[b]					
y-intercept ± error	0.002495 ±0.00015	0.000389 ±0.00047	0.00225 ±0.00034	0.009905 ±0.00045	0.001787 ±0.00036
Slope ± error	0.000229 ±0.0000062	0.000206 ±0.000019	0.000157 ±0.000014	0.000187 ±0.000018	0.000219 ±0.000015
r^2	0.9978	0.9744	0.9772	0.9720	0.9866
x-intercept ± error	-11±0.73	-1.9±2.3	-14±2.5	-53±5.7	-8.2±1.7
ClO_4^- in groundwater ± error, (µg/L)[c]	11±1	2±2	14±3	53±6	8±2

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate was 0.000463 (n = 5). Perchlorate reductase was added to wells in 10 µL of CKB 5/8/2012 extract

[b]Regression parameters are from the regression line for each set (see Figure F-3). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike)

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((\text{slope error/slope})^2 + (\text{y-intercept error/y-intercept})^2)}$
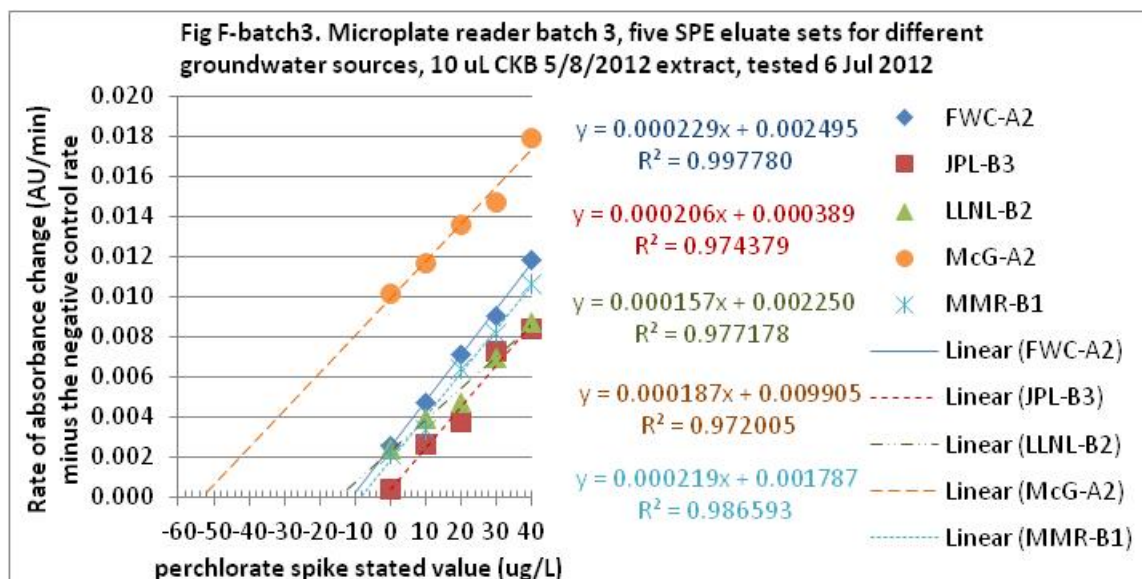

Figure F-3. Perchlorate estimated in plate reader bioassay batch 3 by the standard additions method for SPE eluate sets FWC A2, JPL B3, LLNL B2, NWIRP McGregor A2, and MMR B1, tested 6 Jul 2012
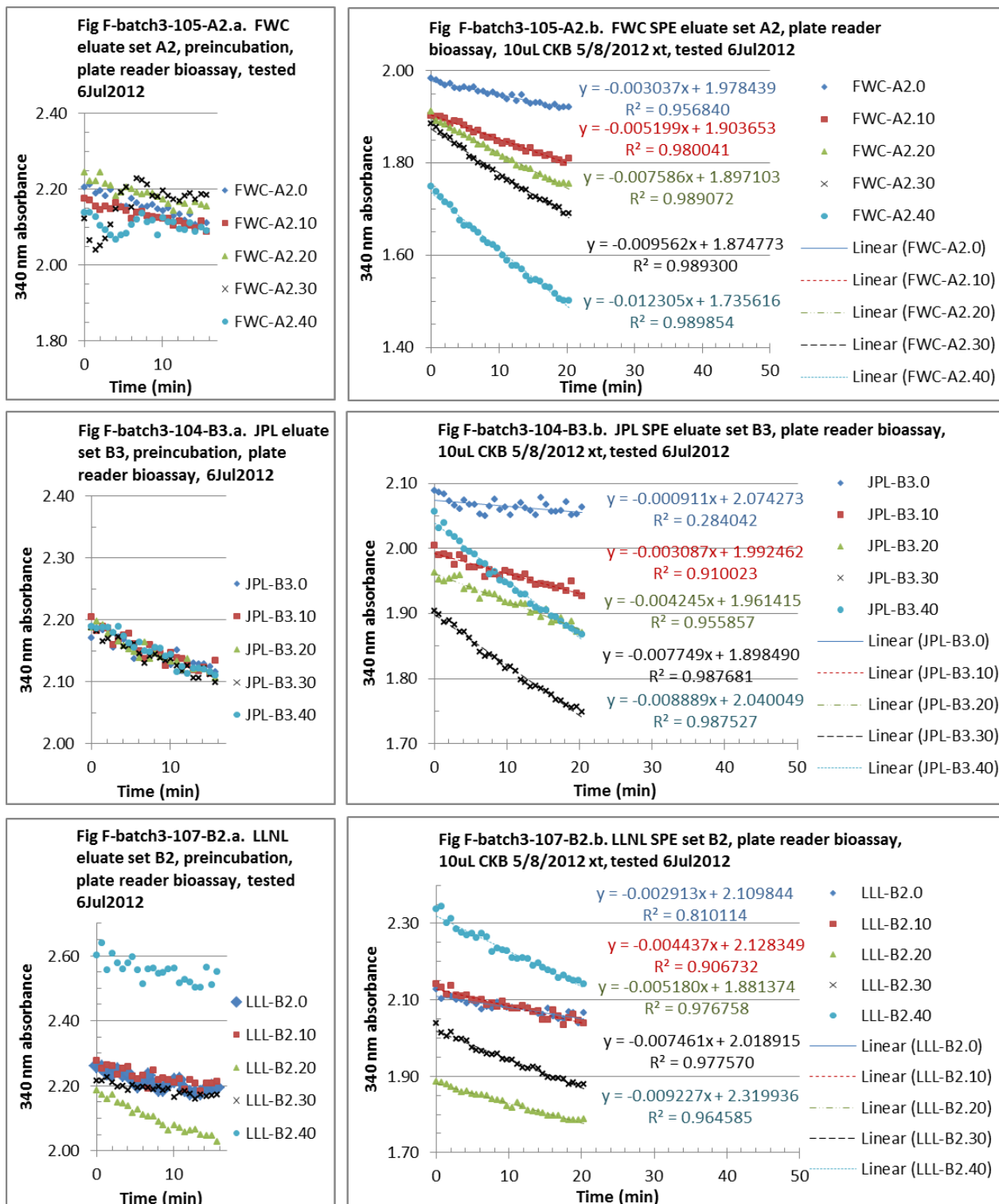
Figure F-3i. Plate reader bioassay batch 3 absorbance change for SPE eluate sets FWC A2, JPL B3, and LLNL B2, 10 μL CKB 5/8/2012 extract, tested 6 Jul 2012
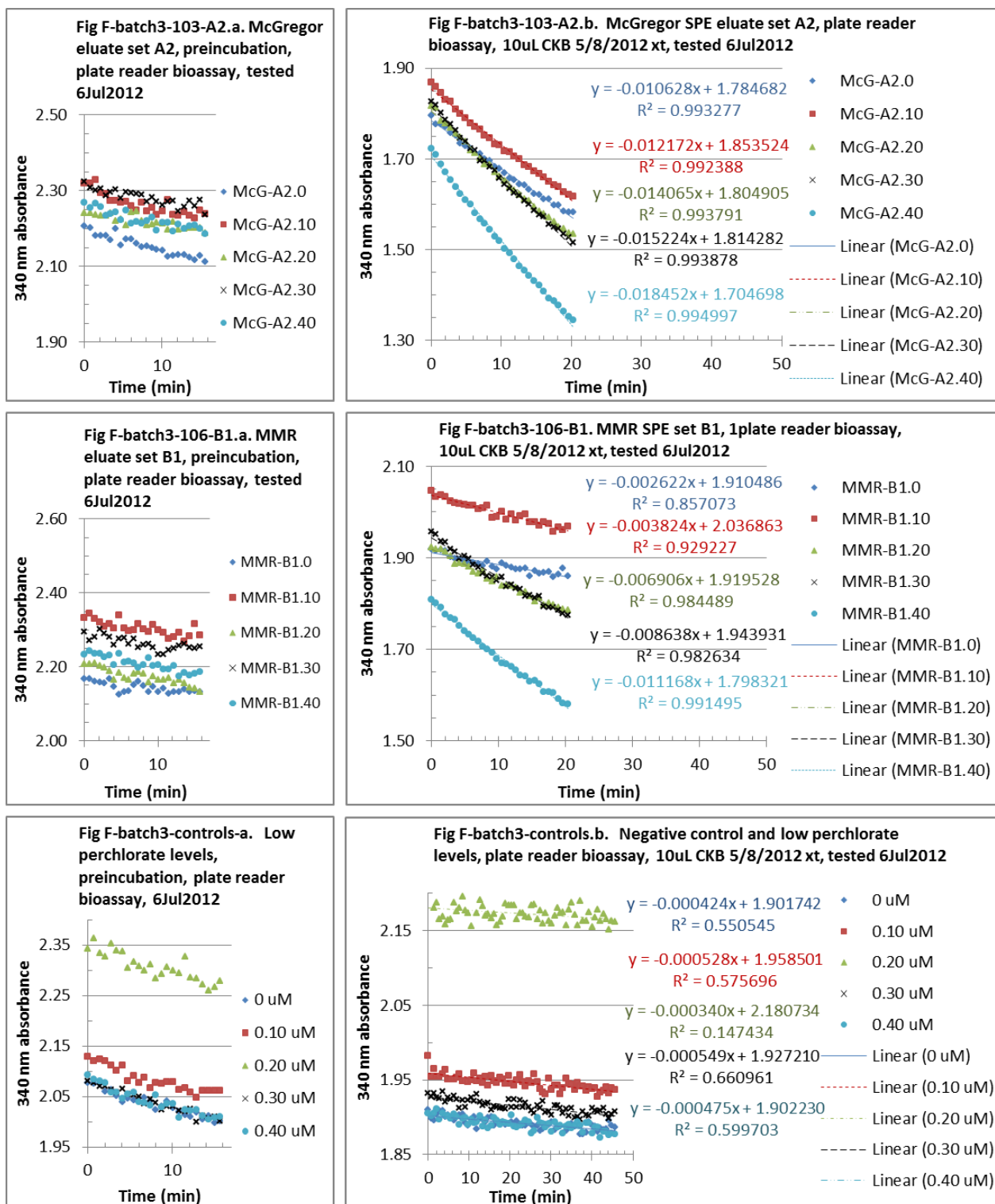
Figure F-3ii. Plate reader bioassay batch 3 absorbance change for SPE eluate sets McGregor A2, MMR B1, and negative control and low perchlorate standards, 10 μL CKB 5/8/2012 extract, tested 6 Jul 2012

Table F-5. Perchlorate estimated in plate reader bioassay batch 4 by the standard additions method for SPE eluate sets FWC-B3, JPL-C2, LLNL-C1, NWIRP McGregor-B2/3, and MMR-C2, tested 11 Jul 2012[a]

Perchlorate Spike (µg/L)	105, FWC-B3	104, JPL-C2	107, LLNL-C1	103, NWIRP McGregor-B2/3[b]	106, MMR-C2	Perchlorate Standards µM	Rate
0	0.001735	-0.000299	0.001726	0.003209	0.000921	0	0
10	0.003507	0.001417	0.002994	0.005764	0.003065	10	0.002746
20	0.004892	0.002903	0.00583	0.007623	0.005078	20	0.006317
30	0.007966	0.004286	0.007298	0.009355	0.007373	30	0.009047
40	0.009083	0.007635	0.010291	0.011832	0.009693	40	0.013132
Regression parameters for each set[c]							
y-intercept ± error	0.001606 ±0.00038	-0.000559 ±0.00050	0.001341 ±0.00040	0.003389 ±0.00021	0.000856 ±0.000087	-0.0002646 ±0.00031	
Slope ± error	0.000192 ±0.000016	0.000187 ±0.000020	0.000214 ±0.000016	0.000208 ±0.0000085	0.000219 ±0.0000035	0.000326 ±0.000013	
r^2	0.9806	0.9656	0.9830	0.9951	0.9992	0.9954	
x-intercept ± error	-8.4±2.1	3.0±2.7	-6.3±1.9	-16±1.2	-3.9±0.40	0.81±0.95	
ClO_4^- in groundwater ± error (µg/L)[d]	8±2	-3±3	6±2	49±4	4±0.4	NA	

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate was 0.000773 (n = 3). Perchlorate reductase was added to wells in 10 µL CKB 5/8/2012 extract

[b]The samples from McGregor groundwater bottle B were diluted 1/3 with deionized water prior to spiking with perchlorate and application to SPE cartridges. Thus the estimated level of perchlorate in groundwater is equal to (3)(-1)(x-intercept) for the B2 eluate set

[c]Regression parameters are from the regression line for each set (see Figure F-4). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[d]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((\text{slope error/slope})^2 + (\text{y-intercept error/y-intercept})^2)}$

Figure F-4. Perchlorate estimated in plate reader bioassay batch 4 by the standard additions method for SPE eluate sets FWC B3, JPL C2, LLNL C1, McGregor B2/3, MMR C2, and control standards, tested 11 Jul 2012

Figure F-4i. Plate reader bioassay batch 4 absorbance change for SPE eluate sets FWC B3, JPL C2, and LLNL C1, 10 μL CKB 5/8/2012 extract, tested 11 Jul 2012

Figure F-4ii. Plate reader bioassay batch 4 absorbance change for SPE eluate sets McGregor B2/3, MMR C2, and perchlorate standards, 10 μL CKB 5/8/2012 extract, tested 11 Jul 2012

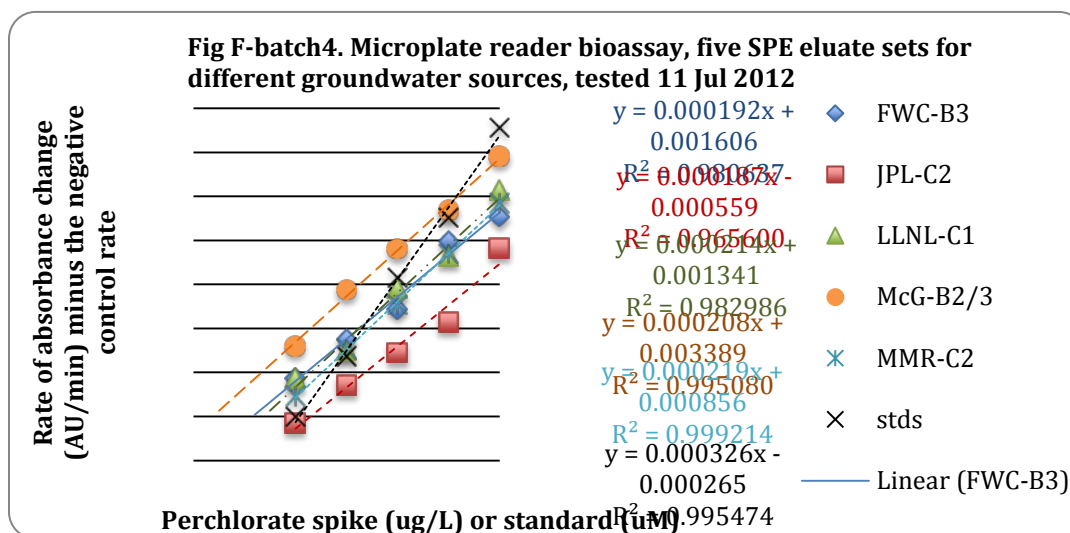**Figure F-4iii. Plate reader bioassay batch 4 absorbance change for negative control wells, 10 μL CKB 5/8/2012 extract, tested 11 Jul 2012**

Table F-6. Perchlorate estimated in plate reader bioassay batch 5 by the standard additions method for SPE eluate sets Hill A2, B1, C3, FWC-C3, and MMR-A2 , tested 11 Jul 2012[a]

Perchlorate Spike (µg/L)	102, Hill-A2	102, Hill-B1	102, Hill-C3	105, FWC-C3	106, MMR-A2	Perchlorate Standards	
						µM	Rate
0	0.004067	0.005696	0.005723	0.002066	0.001068	0	0.000000
10	0.007270	0.005752	0.006529	0.003389	0.003109	10	0.002802
20	0.008763	0.005370	0.007901	0.005620	0.005728	20	0.005926
30	0.009496	0.007739	0.010139	0.007918	0.008759	30	0.008990
40	0.011846	0.010738	0.011760	0.009353	0.011030	40	0.012205
Regression parameters for each set[b]							
y-intercept ± error	0.004732 ±0.00056	0.004645 ±0.0011	0.005274 ±0.00036	0.001849 ±0.00025	0.000824 ±0.00022		-0.000135 ±0.00010
Slope ± error	0.000178 ±0.000023	0.000121 ±0.000044	0.000157 ±0.000014	0.000191 ±0.000010	0.000256 ±0.0000091		0.000306 ±0.0000041
r^2	0.9527	0.7137	0.9750	0.9913	0.9962		0.9995
x-intercept ± error	-27±4.7	-38±17	-34±3.8	-9.7±1.4	-3.2±0.88		0.44±0.33
ClO_4^- in groundwater ± error (µg/L)[c]	27±5	38±17	34±4	10±1	3±1		NA

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the differnt bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate was 0.000941 (n = 2). Perchlorate reductase was added to wells in 10 µL CKB 5/8/2012 extract

[b]Regression parameters are from the regression line for each set (see Figure F-5). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standardl

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((slope\ error/slope)^2 + (y\text{-}intercept\ error/y\text{-}intercept)^2)}$
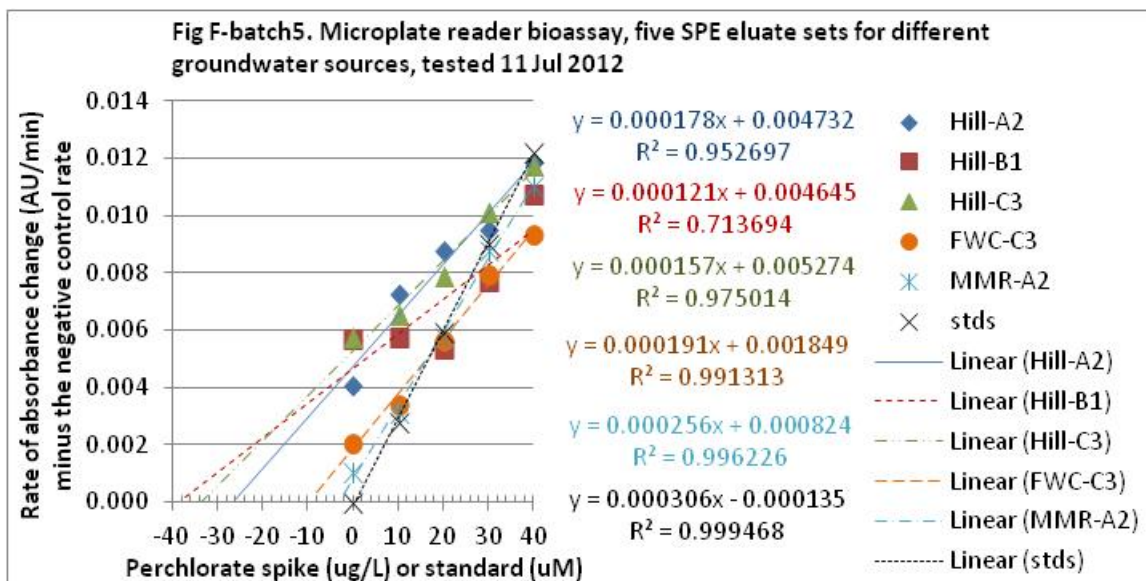
Figure F-5. Perchlorate estimated in plate reader bioassay batch 5 by the standard additions method for SPE eluate sets Hill A2, B1, C3, FWC C3, MMR A2, and control standards, tested 11 Jul 2012
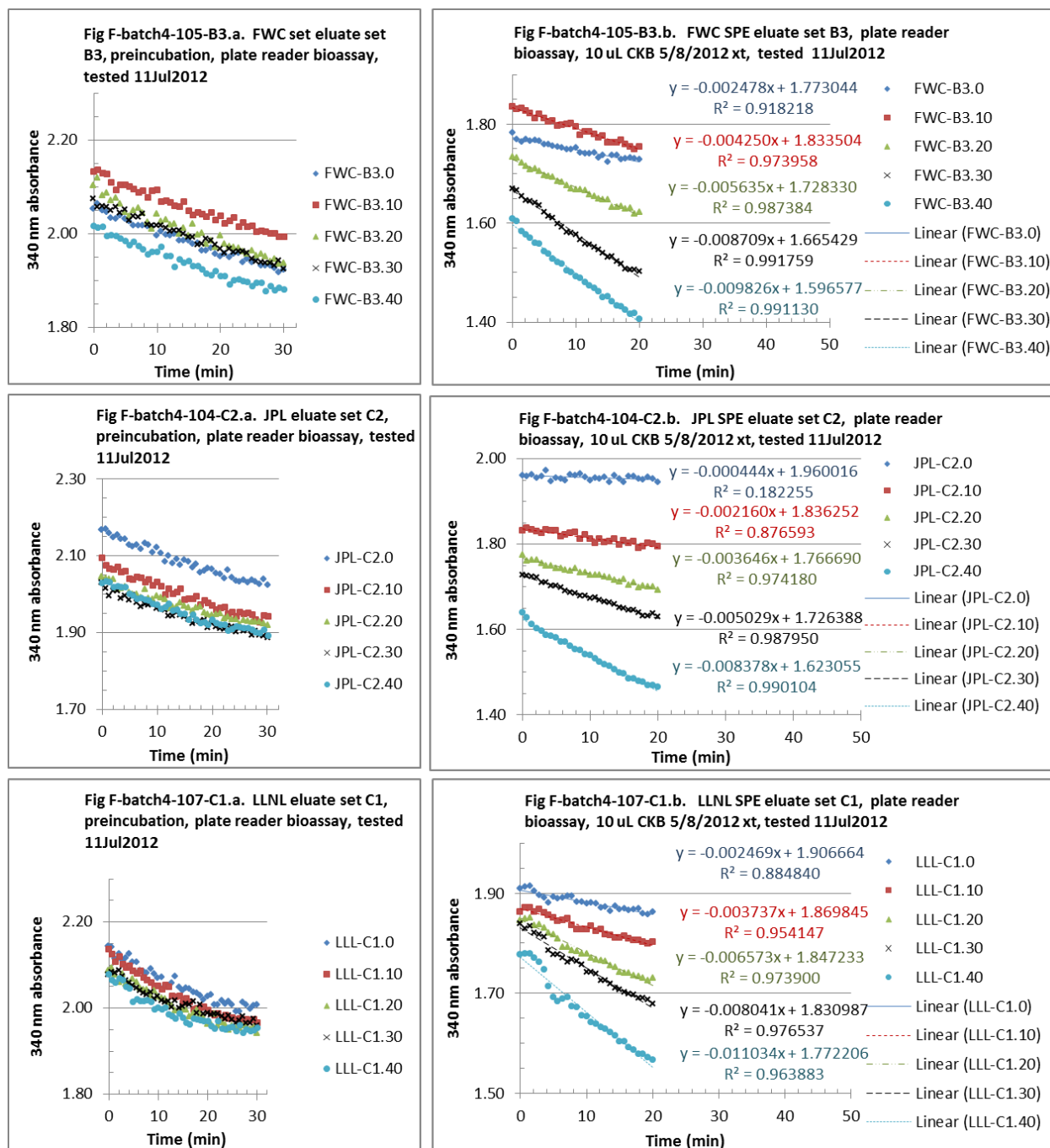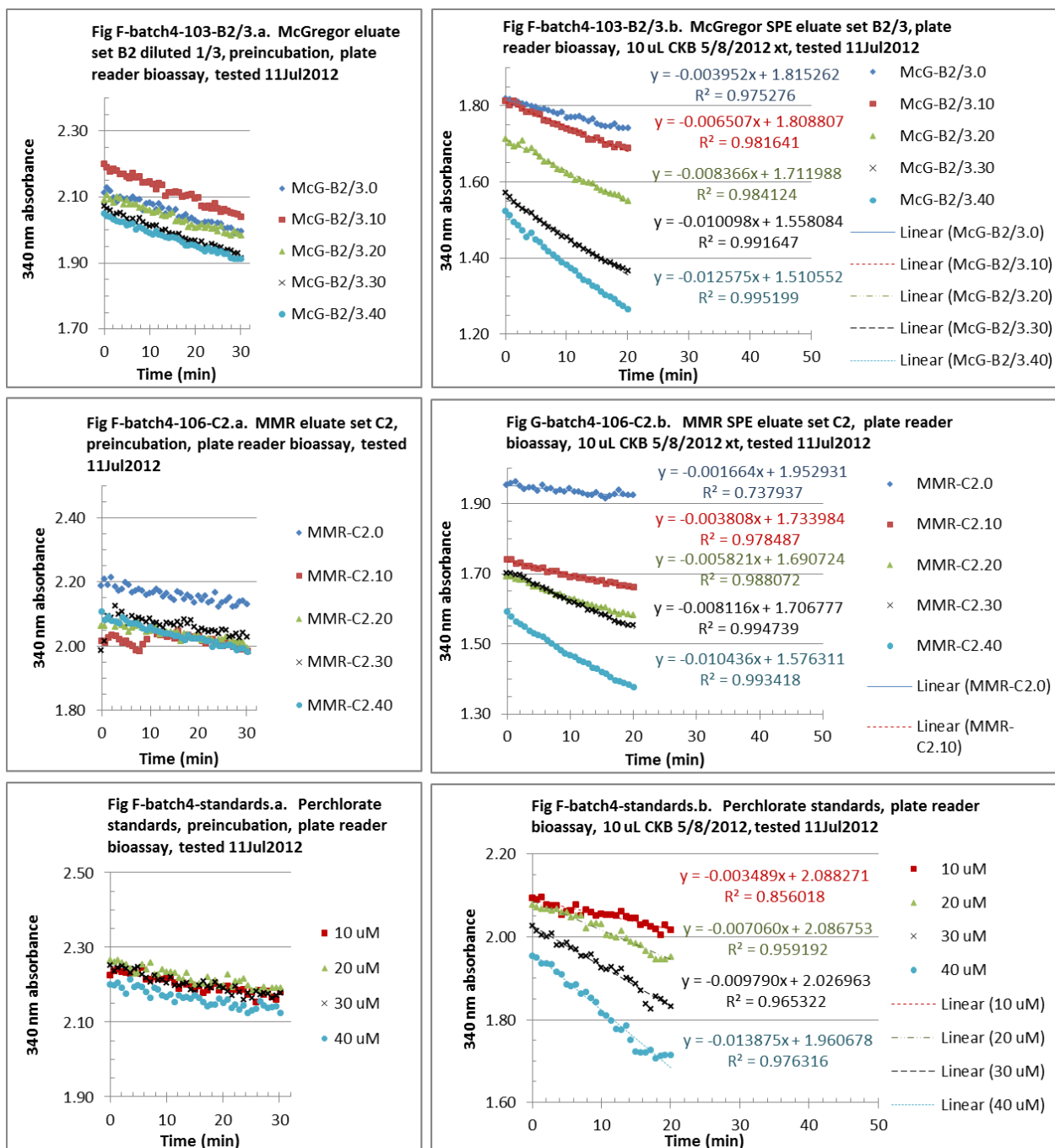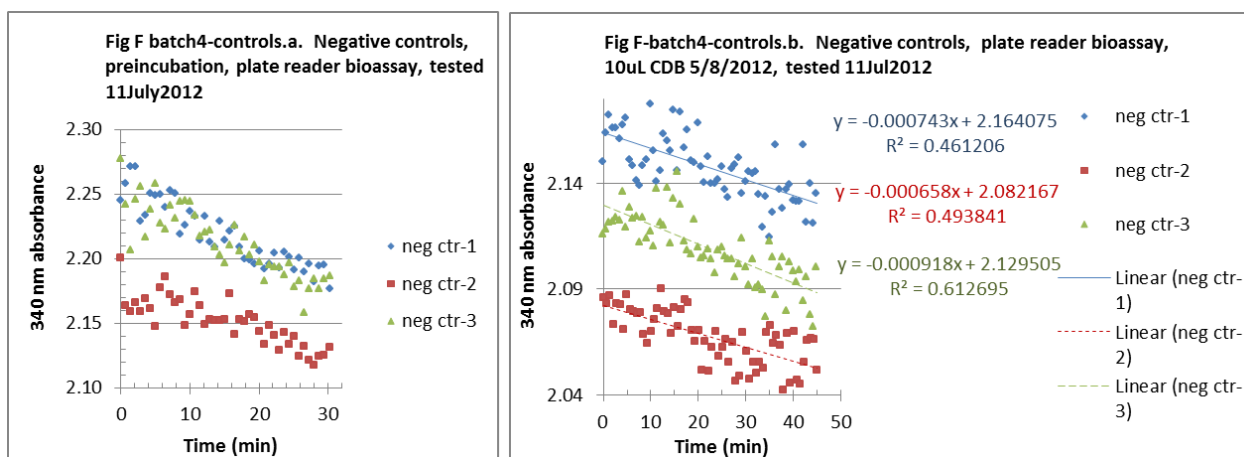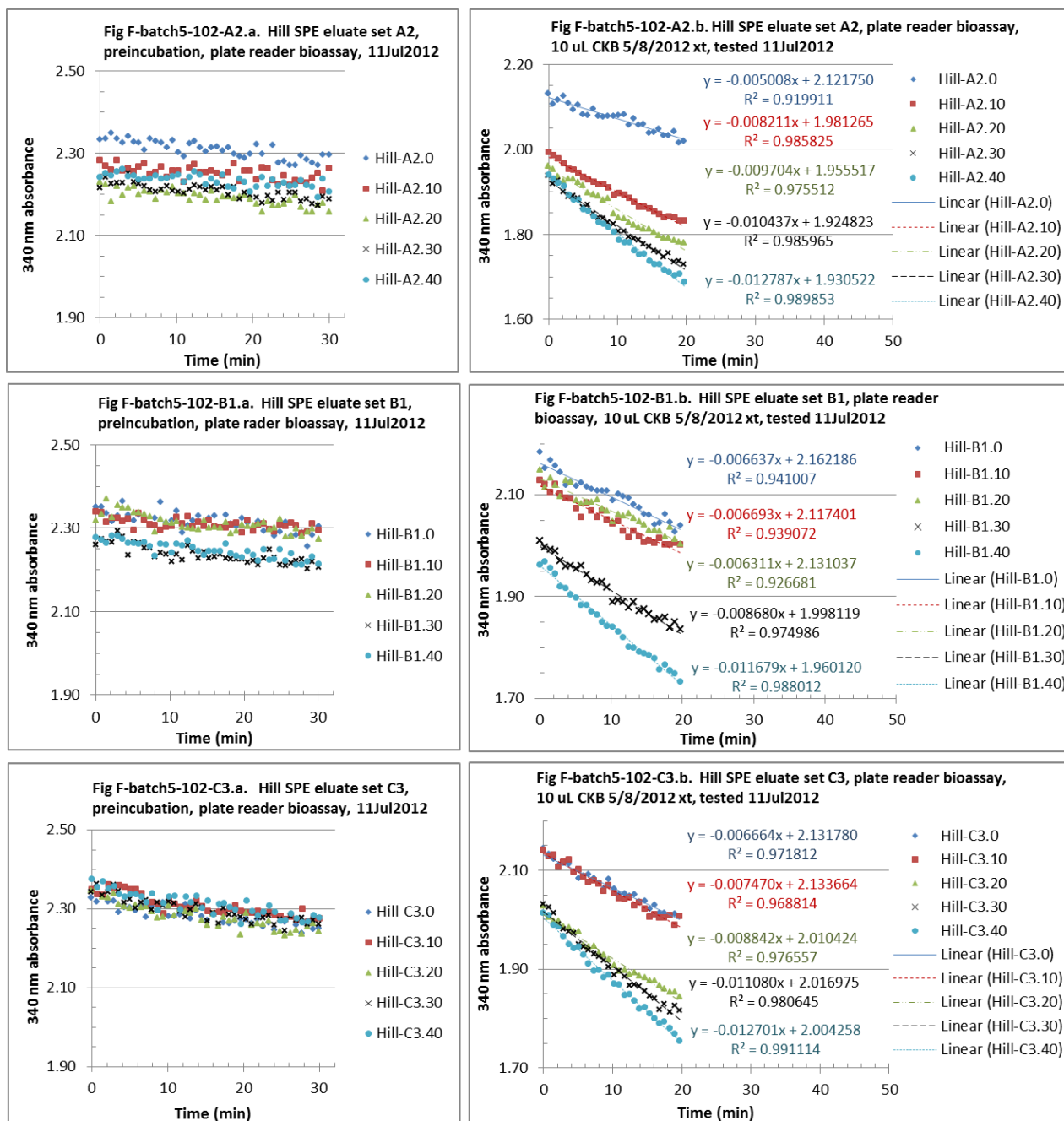
Figure F-5i. Plate reader bioassay batch 5 absorbance change for SPE eluate sets Hill A2, B1, and C3, 10 μL CKB 5/8/2012 extract, tested 11 Jul 2012
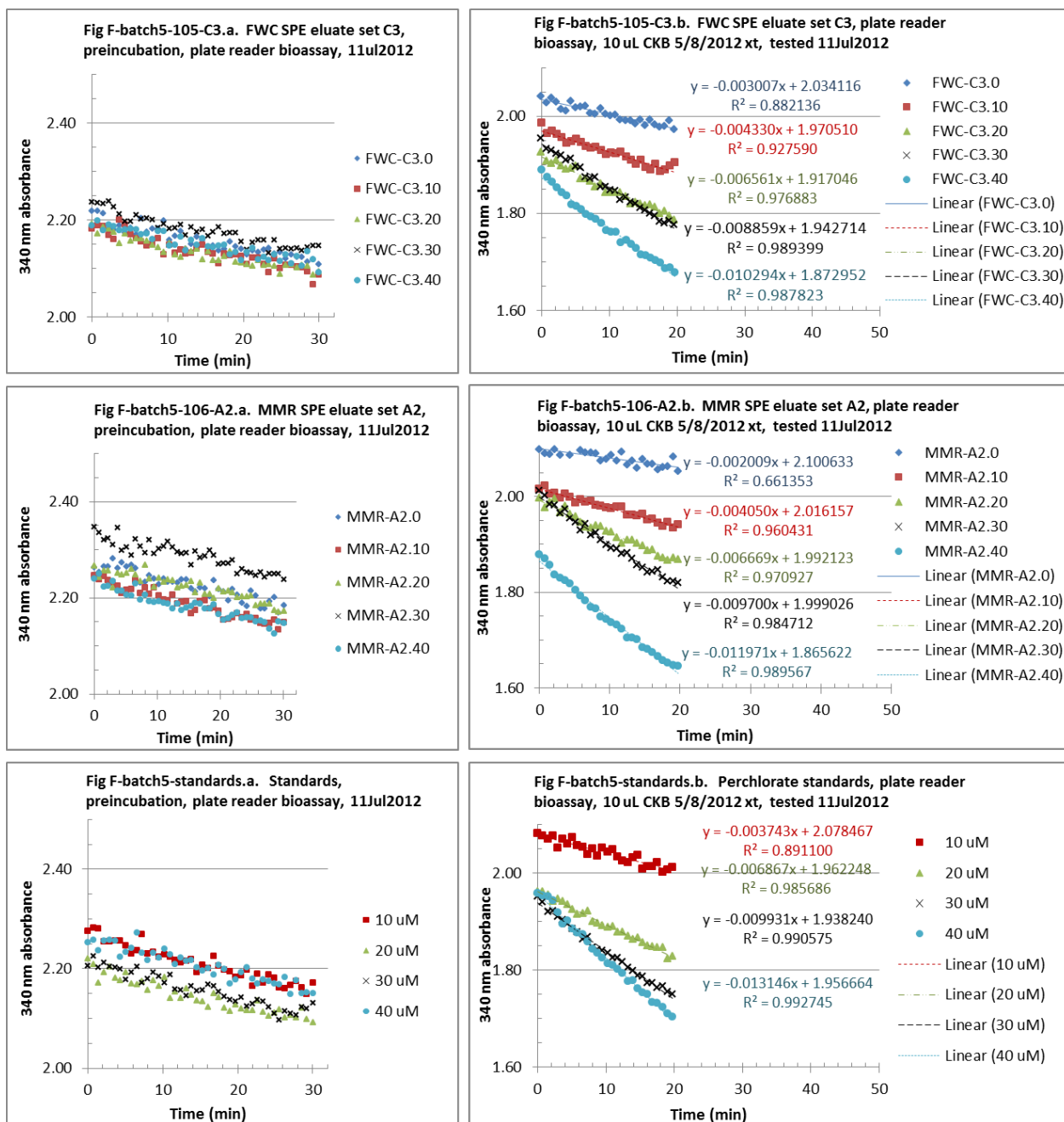
Figure F-5ii. Plate reader bioassay batch 5 absorbance change for SPE eluate sets FWC C3, MMR A2, and perchlorate standards, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012
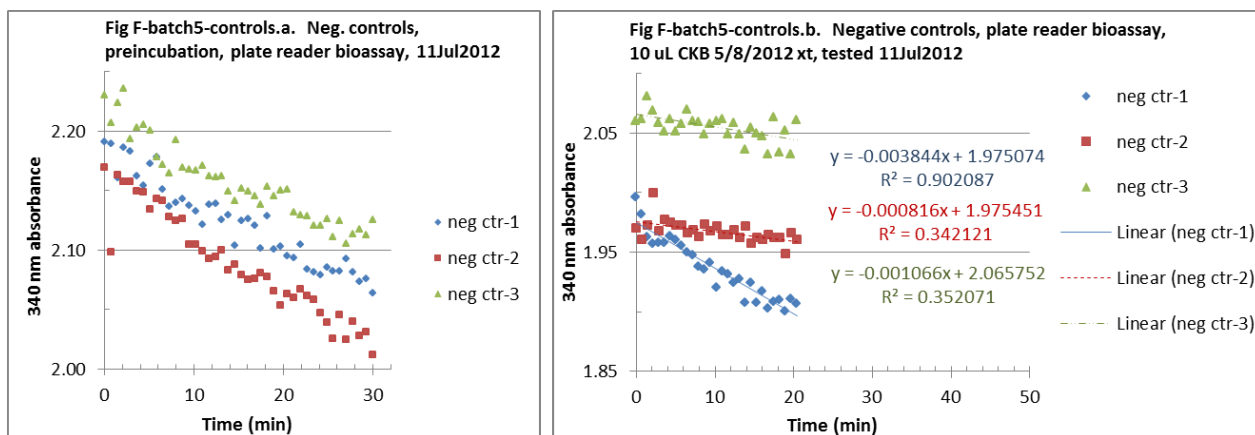
Figure F-5iii. Plate reader bioassay batch 5 absorbance change for negative control wells, 10 μL CKB 5/8/2012 extract, tested 11 Jul 2012

Appendix G: ESTIMATE PERCHLORATE CONCENTRATION BY STANDARD ADDITIONS METHOD PLOTS OF SPE ELUATES ANALYZED BY IC

Benchtop bioassay results were not consistent with historical perchlorate levels for several groundwater sources, including MMR and LLNL (Table 5-4). To help evaluate whether the SPE concentration and purification step had performed according to expectation, six SPE eluate sets were analyzed for perchlorate by IC and the results used for standard additions method estimates of perchlorate concentration in groundwater. Results are listed in Table G-1.

Table G-1. Perchlorate in groundwater estimated by the standard additions method for some SPE eluate sets using data from ion chromatographic determination of perchlorate in eluates[a]

Site	Para-meter[b]	A1	A2	A3	B1	B2	B3	C1	C2	C3	Average ± SD[c]	PD (%)	Histor-ical ClO_4^- (μg/L)
104, JPL	SPE date	8 Dec 2011	nt	nt	nt	nt	nt	nt	nt	nt			<1
	Analysis date	10 Dec 2011	nt	nt	nt	nt	nt	nt	nt	nt			
	μg/L	-1±1	nt	nt	nt	nt	nt	nt	nt	nt	-1	NA	
106, MMR	SPE date	9 Mar 2012	nt	nt	nt	13 Mar 2012	nt	nt	nt	14 Mar 2012			5.8
	Analysis date	28Mar 2012	nt	nt	nt	28Mar 2012	nt	nt	nt	28Mar 2012			
	μg/L	5±1	nt	nt	nt	5±1	nt	nt	nt	5±1	5±0.3	2	
107, LLNL	SPE date	nt	nt	nt	12 Apr 2012	nt	nt	nt	nt	17 Apr 2012			16
	Analysis date	nt	nt	nt	27Apr 2012	nt	nt	nt	nt	27Apr 2012			
	μg/L	nt	nt	nt	20±2	nt	nt	nt	nt	16±4	18±2	38	

[a]nt denotes that there is no data for this cell of the table, PD is the percent difference compared to the reference analytical method performed in a commercial laboratory as listed in Table 5-4
[b]SPE date corresponds to the preparation date for an SPE eluate set. The date of IC analysis is also noted
[c]For MMR eluate sets the spread around the average is given as the standard deviation, for LLNL eluate sets it is the range/2

The IC data for the SPE eluate sets tested from each groundwater source are listed in Tables G-104 (JPL), G-106 (MMR), and G-107 (LLNL). Each of these tables is accompanied by a figure showing the standard additions method regression lines for the SPE eluate sets, from which the perchlorate concentration is determined. The perchlorate spikes added to groundwater prior to the SPE step were 0, 10, 20, 30, or 40 µg/L.

Table G-104. Perchlorate IC analysis of SPE eluate set A1 for Jet Propulsion Laboratory groundwater collected 14 Nov 2011 from location MW-16

Perchlorate Spike (μg/L)	Perchlorate (μM) (IC analysis on 10 Dec 2011) Set A1
0	0
10	6.7
20	16.7
30	25.1
40	35.8
Regression parameters for SPE set[a]	
y-intercept ± error	-1.1273 ± 0.9065
Slope ± error	0.9000 ± 0.0370
r^2	0.9950
x-intercept ± error	1.3±1.0
ClO_4^- in groundwater ± error (μg/L)[b]	1 ± 1

[a]Regression parameters are from the standard additions method regression line for the set (see Figure G-104).
[b]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((slope\ error/slope)^2 + (y\text{-}intercept\ error/y\text{-}intercept)^2)}$

Figure G-104. Perchlorate estimated by the standard additions method for JPL SPE eluate set A1 using data from IC determination of perchlorate in eluates

Table G-106. Perchlorate IC analysis of SPE eluate sets A1, B2, and C3 for Massachusetts Militry Reservation groundwater collected 23 Feb 2012 from location J3-INF

Perchlorate Spike (µg/L)	Perchlorate (µM) (IC analysis on 28 Mar 2012)		
	Set A1	Set B2	Set C3
0(	4.4	3.6	4.7
10	12.7	12.8	13.1
20	20.2	19.8	19.7
30	30.7	27.8	29.3
40	38.3	36.0	37.6
Regression parameters for SPE sets[a]			
y-intercept ± error	4.1012 ± 0.6513	4.0507 ± 0.3999	4.4460 ± 0.5833
Slope ± error	0.8575 ± 0.0266	0.7971 ± 0.0163	0.8214 ± 0.0238
r^2	0.9971	0.9987	0.9975
x-intercept	-4.8±0.77	-5.1±0.51	-5.4±0.73
ClO_4^- in groundwater ± error (µg/L)[b]	5 ± 1	5 ± 1	5 ± 1

[a]Regression parameters are from the standard additions method regression line for a set (see Figure G-106).
[b]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) * $\sqrt{((slope\ error/slope)^2 + (y\text{-}intercept\ error/y\text{-}intercept)^2)}$

Figure G-106. MMR SPE eluate sets A1, B2, C3, IC determination of perchlorate in eluates, analyzed 28 Mar 2012

y = 0.8575x + 4.1012
R^2 = 0.9971

y = 0.7971x + 4.0507
R^2 = 0.9987

y = 0.8214x + 4.446
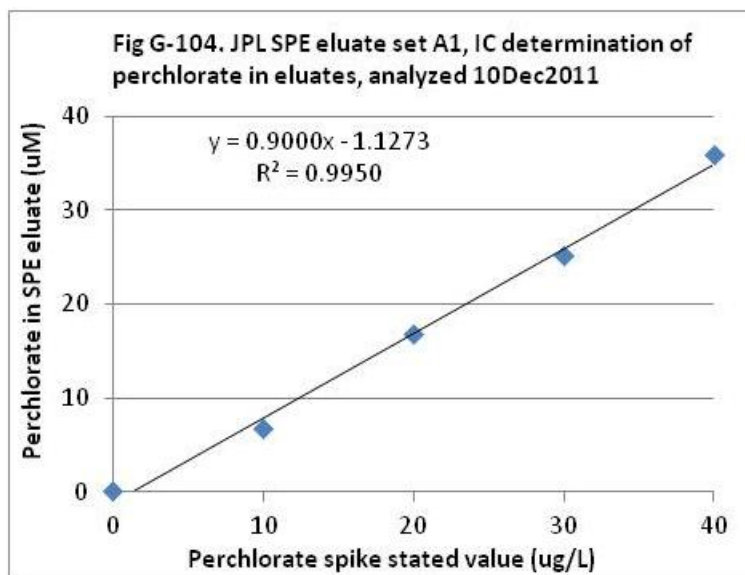R^2 = 0.9975

Figure G-106. Perchlorate estimated by the standard additions method for MMR SPE eluate sets A1, B2, and C3 using data from IC determination of perchlorate in eluates

Table G-107. Perchlorate IC analysis of SPE eluate sets B1 and C3 for Lawrence Livermore National Laboratory groundwater collected 29 Mar 2012 from location W-854-1823

Perchlorate Spike (µg/L)	Perchlorate (µM) (IC analysis on 27 Apr 20120	
	Set B1	Set C3
0	10.9	9.0
10	15.8	19.4
20	24.2	23.3
30	28.3	26.4
40	32.8	37.1
Regression parameters for SPE sets[a]		
y-intercept ± error	11.1333 ± 1.0236	10.4222 ± 2.0007
Slope ± error	0.5633 ± 0.0418	0.6322± 0.0817
r^2	0.9838	0.9523
x-intercept	-20±2.3	-16±3.8
ClO_4^- in groundwater ± error (µg/L)[b]	20 ± 2	16 ± 4

[a]Regression parameters are from the standard additions method regression line for a set (see Figure G-107).
[b]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated ClO_4^- concentration) $* \sqrt{((slope\ error/slope)^2 + (y\text{-}intercept\ error/y\text{-}intercept)^2)}$
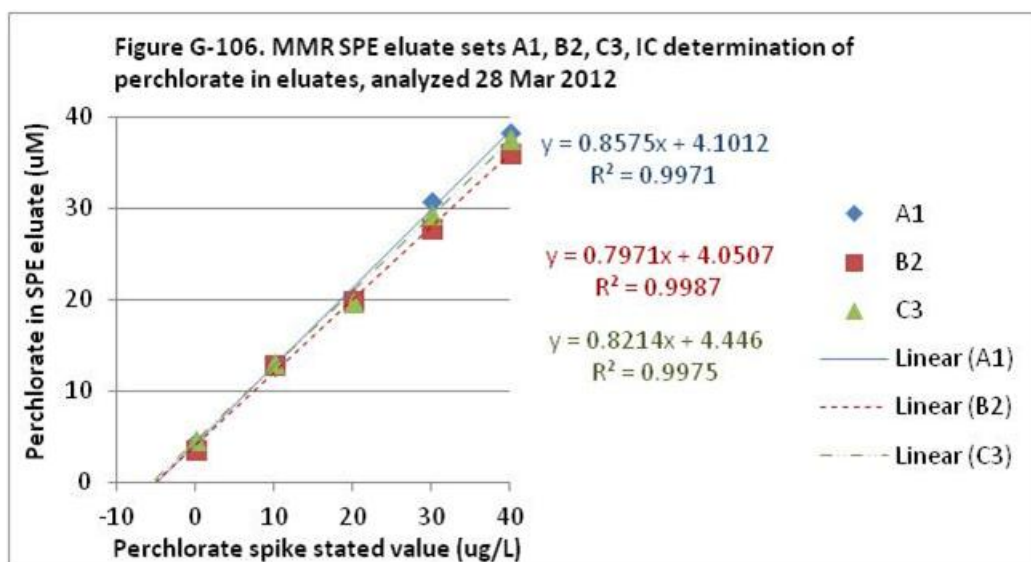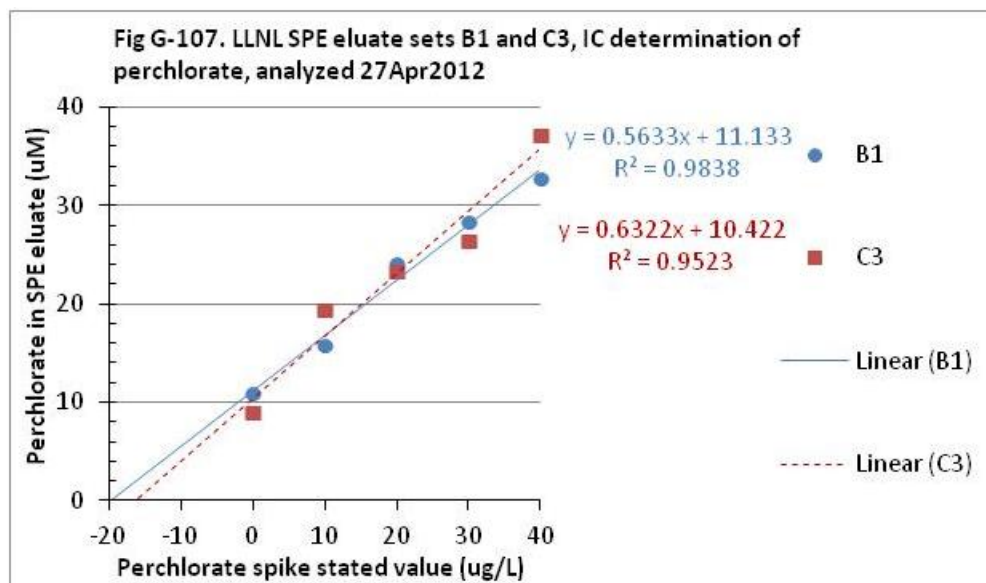

Figure G-107. Perchlorate estimated by the standard additions method for LLNL SPE eluate sets B1 and C3 using data from IC determination of perchlorate in eluates

Appendix H: CHARACTERIZATION OF GROUNDWATER SAMPLES BY ION CHROMATOGRAPHY FOR CHLORIDE, NITRATE, SULFATE, FLUORIDE, NITRITE, BROMIDE, CHLORATE, AND PHOSPHATE

Groundwater was tested for perchlorate by the benchtop bioassay and reference analytical methods, subsequently it was stored 2 – 8 °C. Groundwater samples and a deionized water (i.e. DI) control were tested for other anions by IC on 21 and 23 May 2012. See Table H-1.

Table H-1. Anions in groundwater sources or deionized water by ion chromatography

Anion	102, Hill (mg/L)	103A, McGregor (mg/L)	103B, McGregor (mg/L)	104, JPL (mg/L)	105, FWC (mg/L)	106, MMR (mg/L)	107, LLNL (mg/L)	DI control (mg/L)
Chloride	51	27	28	47	11	11	157	<1
Nitrate	11	21	18	6	32	1	24	<1
Sulfate	31	81	90	42	15	6	19	<1
Fluoride	<0.4	0.6	0.8	0.8	<0.2	<0.2	<0.6	<0.2
Nitrite	<2	<2	<2	<2	<1	<1	<3	<1
Bromide	<2	<2	<2	<2	<1	<1	<3	<1
Chlorate	<0.8	<0.8	<0.8	<0.8	<0.4	<0.4	<1.2	<0.4
Phosphate	<4	<4	<4	<4	<2	<2	<6	<2

Groundwater samples and a DI control (i.e. LRB) were applied to SPE cartridges according to the standard protocol on 4 Jun 2012, and the effluents were analyzed by IC for anions on 8 Jun 2012. Results are listed in Table H-2. The anions listed were not extracted by SPE cartridges.

Table H-2. Anions in SPE effluents by ion chromatography

Anion	102, Hill (mg/L)	103A, McGregor (mg/L)	103B, McGregor (mg/L)	104, JPL (mg/L)	105, FWC (mg/L)	106, MMR (mg/L)	107, LLNL (mg/L)	LRB control (mg/L)	Extracted by SPE cartridge
Chloride	52	25	26	48	11	11	155	<1	No
Nitrate	11	20	18	7	29	<1	28	<1	No
Sulfate	28	79	88	39	13	4	17	<1	No
Fluoride	0.3	0.4	0.5	0.6	0.3	<0.2	0.6	<0.2	No
Nitrite	<1	<1	<1	<1	<1	<1	<3	<1	No
Bromide[a]	9	9	9	9	9	9	10	6	NA
Chlorate	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<1.2	<0.4	No
Phosphate	<2	<2	<2	<2	<2	<2	<6	<2	No

[a]SPE cartridges were conditioned with decyltrimethyl ammonium bromide (DTAB). It is likely that residual DTAB in the cartridges was responsible for the bromide seen in the SPE effluents

Appendix I: ICPMS CHARACTERIZATION OF GROUNDWATER SOURCES AND SPE ELUATES

Groundwater sources and freshly prepared SPE eluates from groundwaster sources were analyzed by ICPMS to characterize the pattern of elements present. It was anticipated that this information might provide insight into factors that could impact the performance of the benchtop bioassay. Results of the groundwater testing are listed in Table I-1, and of the SPE eluates in Table I-2.

Table I-1. Elements detected in groundwater samples by ICPMS (5 Jun 2012)

Row No.	Element	102, Hill (µg/L)	103A, McGregor (µg/L)	103B, McGregor (µg/L)	104, JPL (µg/L)	105, FWC (µg/L)	106, MMR (µg/L)	107, LLNL (µg/L)	Deion-ized water (µg/L)
1	Li	21.989	16.974	21.701	3.329	2.836	0.699	96.808	0.001
2	B	37.424	112.275	136.568	43.066	0	0	1518.960	0.217
3	Na	28606.923	31726.54	34541.261	31284.948	17149.138	7009.164	124190.113	63.53
4	Mg	23497.595	4196.883	5411.412	19987.998	9069.109	1516.18	14867.993	0.16
5	Al	0	0	0	3.744	0	2.198	0	0.069
6	Si	8935.548	6510.043	6930.117	13351.408	8834.639	3185.705	17060.435	0.505
7	K	4885.533	1373.443	1427.469	3154.717	1598.434	541.295	7418.409	3.197
8	Ca	21105.449	34760.428	37101.644	40566.559	34990.280	1641.695	24419.616	2.893
9	Sc	1.710	0.710	0.729	2.953	1.626	0	4.280	0
10	Ti	0	0.555	1.294	1.479	0.370	0.555	0.370	0.023
11	V	0	0	0	11.244	6.052	0	5.693	0.011
12	Cr	16.961	0	0	0	0	0	0	0.071
13	Mn	0	0	0	0	0	0.287	0.262	0.001
14	Fe	513.072	585.614	465.305	526.120	388.798	388.909	523.356	0
15	Co	0	0	0	0	0	0	0	0.001
16	Ni	0	0	0	0	0	0.777	0	0.014
17	Cu	0	0	0	0	0	2.032	1.058	0.003
18	Zn	0	0	0	0	0	0	0	4.76
19	Ga	0	0	0	0	0	0	0	0.002
20	As	0.621	0.078	0.466	4.504	0.544	0.155	3.960	0.005
21	Br	0	77.617	198.063	0	49.068	53.083	670.535	7.628
22	Rb	0.783	1.539	0	0.047	0.116	0.951	12.154	0
23	Sr	219.569	400.705	516.038	470.656	295.742	18.403	762.416	0
24	Y	0	0	0	0	0	0.005	0.021	0
25	Zr	0.052	0.080	0	0.068	0.104	0.057	0.052	0
26	Nb	0	0	0	0	0	0	0.006	0
27	Mo	6.141	1.175	1.668	3.944	4.146	0.251	9.412	0
28	Ru	0	0	0	0	0	0	0	0
29	Rh	0	0	0	0	0	0	0	0
30	Pd	0	0	0	0	0	0	0	0
31	Cd	0	0	0	0	0	0	0	0
32	In	0	0	0	0	0.031	0.013	0.018	0
33	Sn	0	0	0	0	0	0	0	0
34	Sb	0	0	0	0	0	0	0	0
35	Cs	0.013	0.027	0.017	0.01	0.003	0.003	0.094	0
36	Ba	141.769	40.520	38.967	82.818	44.902	3.289	53.018	0.143

Table I-1, continued

Row No.	Element	102, Hill (µg/L)	103A, McGregor (µg/L)	103B, McGregor (µg/L)	104, JPL (µg/L)	105, FWC (µg/L)	106, MMR (µg/L)	107, LLNL (µg/L)	Deion-ized water (µg/L)
37	La	0	0	0	0	0	0	0	0
38	Ce	0	0	0	0	0	0	0	0
39	Pr	0	0	0	0	0	0	0	0
40	Nd	0	0	0	0	0	0	0	0
41	Sm	0	0	0	0	0	0	0.034	0
42	Eu	0.010	0	0	0	0	0	0	0
43	Gd	0	0	0	0	0	0	0	0
44	Tb	0	0	0	0	0	0	0	0
45	Dy	0	0	0	0	0	0	0	0
46	Ho	0.020	0.004	0.004	0.008	0.012	0.020	0.016	0
47	Er	0.000	0	0	0	0	0	0	0
48	Tm	0	0	0	0	0	0	0	0
49	Yb	0	0	0	0	0	0	0	0
50	Lu	0	0.003	0	0	0	0	0	0
51	Ir	0.619	0.319	0.480	0.491	0.788	0.263	0.726	0.001
52	Pt	0	0	0	0	0	0	0	0
53	Tl	0.042	0.084	0.092	0	0.127	0	0.148	0
54	Pb	0	0	0	0	0	0.086	0.057	0
55	Bi	0	0	0.005	0.005	0.010	0	0.010	0
56	Th	0.185	0.168	0.129	0.101	0.236	0.056	0.247	0
57	U	11.468	3.121	3.904	7.566	3.53	0	26.115	0.003

Table I-2. Elements detected in SPE eluate samples by ICPMS (5 Jun 2012)[a]

Row No.	Element	102, Hill (µg/L)	103A, Mcgregor (µg/L)	103B, Mcgregr (µg/L)	104, JPL (µg/L)	105, FWC (µg/L)	106, MMR (µg/L)	107, LLNL (µg/L)	LRB Eluate (µg/L)[b]	MOPS – NaCl solution (µg/L)
1	Li	13	12	13	12	9	12	11	8	13
2	B	5021	4605	4761	4718	4673	4849	5315	4854	4856
3	Na	S	S	S	S	S	S	S	S	S
4	Mg	39	22	83	25	26	20	30	18	12
5	Al	1895	1834	1843	1856	1849	1767	1869	1897	1812
6	Si	53263	51783	52544	69028	71741	68053	72420	71895	51231
7	K	2439	2317	2325	2036	2069	1962	2247	2068	2388
8	Ca	1412	1111	1209	2497	2752	2511	2935	2467	1080
9	Sc	19	16	18	22	20	21	22	19	18
10	Ti	136	130	140	150	146	143	148	136	206
11	V	176	169	170	166	161	159	173	155	173
12	Cr	184	59	65	838	918	794	894	824	81
13	Mn	0.65	0.25	0.12	0.22	0.34	0.11	0.115	0	0.43
14	Fe	94	56	55	31	0	2	0	0	0
15	Co	0.44	0.86	0.51	0.90	0	0.25	1.85	0	0.14
16	Ni	0	0	0.042	0	0	0	0	0	0
17	Cu	21	18	15	16	16	19	34	11	17

Table I-2, continued

Row No.	Element	102, Hill (µg/L)	103A, Mcgregor (µg/L)	103B, Mcgregor (µg/L)	104, JPL (µg/L)	105, FWC (µg/L)	106, MMR (µg/L)	107, LLNL (µg/L)	LRB Eluate (µg/L)[b]	MOPS – NaCl solution (µg/L)
18	Zn	0	0	0	10	0	0	0	0	0
19	Ga	0.57	0.56	0.48	0.32	0.48	0.41	0.38	0.29	0.37
20	As	116	117	114	116	107	110	111	108	130
21	Br	724983	720631	736332	197877	183996	198868	190967	210507	7735
22	Rb	1.95	1.87	1.86	2.16	1.87	1.92	1.88	2.00	1.63
23	Sr	12	10	11	9	8	11	11	9	12
24	Y	0.005	0	0	0.021	0	0.121	0	0.016	0.011
25	Zr	6	5	5	4	4	3	4	2	4
26	Nb	0.064	0.11	0.045	0.077	0.155	0.064	0.116	0.084	0.148
27	Mo	45	6	7	16	22	4	39	2	0
28	Ru	0	0	0	0	0	0	0	0	0
29	Rh	0	0	0	0	0	0	0	0	0
30	Pd	0	0.021	0	0	0.021	0	0	0	0
31	Cd	0.035	0	0.039	0.079	0.035	0	0	0.040	0
32	In	0.004	0	0	0.004	0.004	0	0	0	0
33	Sn	0	0	0	0	0	0	0	0	0
34	Sb	0	0	0	0	0	0	0	0	0
35	Cs	0.074	0.074	0.043	0.05	0.05	0.04	0.063	0.053	0.053
36	Ba	34	23	23	22	24	21	25	23	23
37	La	0.004	0	0	0.03	0.03	0.013	0.017	0	0
38	Ce	0	0	0	0	0	0.041	0.157	0	0
39	Pr	0	0	0	0	0	0	0	0	0
40	Nd	0	0	0	0	0	0	0	0	0
41	Sm	0	0	0	0	0.025	0	0	0	0
42	Eu	0	0	0	0	0	0	0	0.009	0
43	Gd	0	0	0	0	0	0	0	0	0
44	Tb	0	0	0	0	0	0	0	0	0.002
45	Dy	0	0	0	0	0	0.008	0	0	0
46	Ho	0.008	0.008	0	0	0.02	0.012	0.004	0.028	0.024
47	Er	0	0	0	0	0	0	0	0	0
48	Tm	0	0	0	0	0	0	0	0	0
49	Yb	0	0	0	0	0	0	0	0	0
50	Lu	0	0	0	0	0	0	0	0	0
51	Ir	0.078	0.056	0.075	0.300	0.338	0.206	0.257	0.131	0.075
52	Pt	0	0	0	0	0.026	0.027	0	0.104	0
53	Tl	0.034	0.035	0.028	0.067	0.035	0.028	0.084	0.141	0.202
54	Pb	1.30	1.07	0.94	0.97	1.48	0.95	1.10	0.91	2.36
55	Bi	0	0	0	0	0.01	0	0	0.005	0.005
56	Th	0	0	0	0	0.073	0	0	0.073	0
57	U	707	190	178	501	262	0	1779	0	0

[a]SPE eluates were prepared 4 Jun 2012 and stored at 2-8 °C until shortly before ICPMS analysis the next day. 200 mL of groundwater were applied to an SPE cartridge, rinsed, and eluted with alkaline MOPS-NaCl solution. Groundwater applied to SPE cartridges was from retains that had been stored at 2-8 °C since the preparation of sample aliquots for perchlorate testing by bioassay and reference analytical methods: Hill (B-retain), McGregor (A and B-retains), JPL (A-retain), FWC (B-retain), MMR (A-retain), and LLNL (A-retain). "S" indicates element was above the range of concentration for analysis.

[b]The LRB eluate was obtained by applying 200 mL of deionized water to an SPE cartridge and eluting with the alkaline MOPS-NaCl solution according to the standard procedure.

The elements that had the highest variation in concentration among the different SPE eluates were considered possible candidates for impacting bioassay performance. The amounts of these in groundwater and SPE eluates were analyzed by non-metric multidimensional scaling (MDS). The MDS analysis indicated that five elements were concentrated in SPE eluates relative to their levels in groundwater (see Figure I-4) while 3 were higher in groundwater compared to their levels in SPE eluates (see Figure I-5). Table I-3 shows the μM concentration of these elements in SPE eluates. Also shown are the concentrations in the LRB eluate as well as the alkaline MOPS-NaCl solution used to elute the SPE cartridges.

Table I-3. Elements concentrated in SPE eluates

Row No.[a]	Element	102, Hill (μM)	103A, Mcgregor (μM)	103B, Mcgregor (μM)	104, JPL (μM)	105, FWC (μM)	106, MMR (μM)	107, LLNL (μM)	LRB Eluant (μM)	MOPS - NaCl solution (μM)
12	Cr	3.5	1.1	1.3	16	18	15	17	15.8	1.6
15	Co	0.0075	0.015	0.0087	0.015	0	0.0042	0.031	0	0.002
17	Cu	0.33	0.23	0.24	0.25	0.25	0.30	0.54	0.17	0.27
27	Mo	0.47	0.063	0.073	0.17	0.23	0.042	0.41	0.02	0
57	U	3.0	0.80	0.75	2.1	1.1	0	7.5	0	0

[a]The row number refers to the row numbers used in Tables I-1 and I-2

The data shown in Table I-3 suggested that the relatively high uranium in the LLNL eluate might correlate with the interference effect noted in the benchtop bioassay with SPE eluates from this source. With respect to the other elements shown in Table I-3, cobalt levels were low (i.e. 0.031 μM or less) from all groundwater sources and thus less likely to impact bioassay performance. Copper levels were the same order of magnitude in the alkaline MOPS-NaCl solution as in SPE eluates and thus not likely to be a factor correlated with the difference in benchtop bioassay performance observed between LLNL SPE eluates, for which interference occurred, and the enzyme control reactions tested in the same batches where no interference was seen (see Figure B-107). Molybdenum concentrations showed about an 11-fold difference in concentration among the SPE eluates, however, molybdenum concentrations weren't very different for the Fontana Water Co. (FWC) and LLNL eluates. In benchtop bioassay testing, the results with FWC eluates were in better agreement with reference analytical methods than for LLNL eluates (Table 5-4). Chromium levels were high in many eluates (i.e. 16 to 18 μM), but since levels were high with both FWC and LLNL, chromium seems unlikely to correlate with the difference in benchtop bioassay performance between them.

The capacity of SPE cartridges employed in this project to concentrate uranium is shown in Table I-4 and Figure I-1. Removal of uranium from groundwater by ion exchange processes is a well described phenomenon[23], and so finding that uranium is concentrated in SPE eluates is not an unexpected observation for the SPE processing step. The average percent recovery of uranium in SPE eluates ± standard deviation was 68 ± 10. In addition, several SPE eluate sets for LLNL, FWC, and Hill, which had previously been tested for perchlorate by the benchtop bioassay, were analyzed for uranium by ICPMS (Table I-5 and Figure I-2). The uranium content of these older SPE eluates was similar to that presented in Table I-4. For comparison, the SPE eluates and

groundwater sources examined by ICPMS were also analyzed by IC for perchlorate (Table I-6, and Figure I-3). The recovery of perchlorate in SPE eluates ranged from 39 to 96 percent.

Table I-4. Comparison of uranium in groundwater sources and SPE eluates

Sample Name	Groundwater		SPE eluates				
	Concen-tration[a] (µg/L)	Concen-tration normalized to LLNL	Concen-tration[b] (µg/L)	Concen-tration normalized to LLNL	Percent uranium recovered in eluate	Concen-tration factor	Concen-tration[c] (µM)
107, LLNL	26	1.00	1779	1.00	68	68	7.5
102, Hill AFB	11	0.42	707	0.40	64	64	3.0
104, JPL	7.6	0.29	501	0.28	66	66	2.1
105, FWC	3.5	0.13	262	0.15	75	75	1.1
103, Mcgregor-A	3.1	0.12	190	0.11	61	61	0.8
103, McGregor-B	3.9	0.15	178	0.10	46	46	0.8
106, MMR	0	0.00	0	0.0001	NA	NA	0.0008

[a]Data are from row 57 of Table I-1
[b]Data are from row 57 of Table I-2
[c]Data are from Table I-3
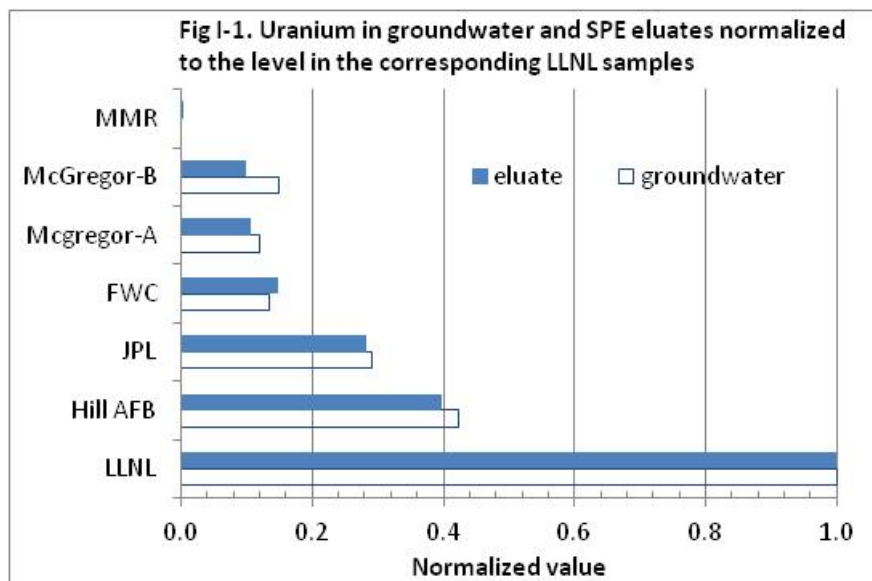
Figure I-1. Uranium detected in groundwater and SPE eluates by ICPMS (5 Jun 2012)

Table I-5. Uranium detected by ICPMS (24 Jul 2012) in some SPE eluate sets previously tested by benchtop bioassay for perchlorate

SPE eluate source	ClO$_4^-$ spike (µg/L)	Uranium in SPE eluates (µg/L)					Pooled average for uranium in SPE eluates[a]		
							(µg/L)	Normalized to LLNL	(µM)
107, LLNL		B2	C1	B3	C3	Replicate C3			
	0	1,892	2,121	2,046	2,099	2,094	2076 ±42	1.00±0.02	8.7 ±0.16
	10	2,542	2,022	2,158	2,207	2,191			
	20	1,599	2,133	2,062	2,087	2,064			
	30	2,034	1,935	2,207	2,060	2,036			
	40	1,980	2,097	2,053	2,113	2,080			
	Average ±SD	2,009 ±342	2,062 ±83	2,105 ±73	2,113 ±56	2,093 ±59			
105, FWC		A2	B1						
	0	297	292				292 ±3	0.14±0.002	1.2 ±0.01
	10	292	277						
	20	296	298						
	30	284	284						
	40	305	291						
	Average ±SD	295 ±8	288 ±8						
102, Hill AFB		C3	B3						
	0	761	737				735 ±23	0.35±0.01	3.1 ±0.10
	10	760	651						
	20	760	682						
	30	776	792						
	40	732	696						
	Average ±SD	758 ±16	712 ±55						

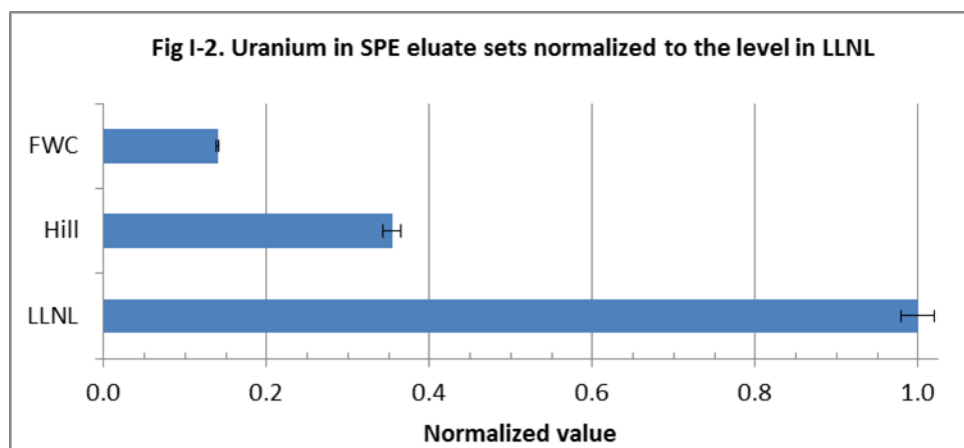[a]The spread about the average is ± standard deviation for LLNL, or ± range/2 for FWC and Hill

Figure I-2. Uranium detected by ICPMS (24 Jul 2012) in some SPE eluate sets previously tested by benchtop bioassay for perchlorate. Pooled averages for the SPE eluate sets from the three groundwater sources were normalized to the LLNL pooled average. The error bars correspond to the standard deviation (LLNL) or the range/2 (FWC and Hill)

Table I-6. Perchlorate detected by IC in groundwater and SPE eluates[a]

Sample Name	Groundwater		SPE eluates			
	Concentration (µg/L)	Concentrations normalized to McGregor-A	Concentration (µg/L)	Concentrations normalized to McGregor-A	Percent perchlorate recovered in eluate	Concentration factor
107, LLNL	14	0.23	1060	0.40	76	76
102, Hill AFB	36	0.59	1720	0.65	48	48
104, JPL	NA	NA	NA	NA	NA.	NA
105, FWC	12	0.20	910	0.35	76	76
103, McGregor-A	61	1.00	2630	1.00	43	43
103, McGrebor-B	53	0.87	2060	0.78	39	39
106, MMR	7	0.11	670	0.25	96	96

[a]SPE eluates were prepared 4 Jun 2012. Groundwater and these SPE eluates had also been analyzed by ICPMS to characterize the elements present (see Tables I-1, I-2, I-3, I-4, and Fig. I-1). Storage was at 2-8 °C prior to IC testing
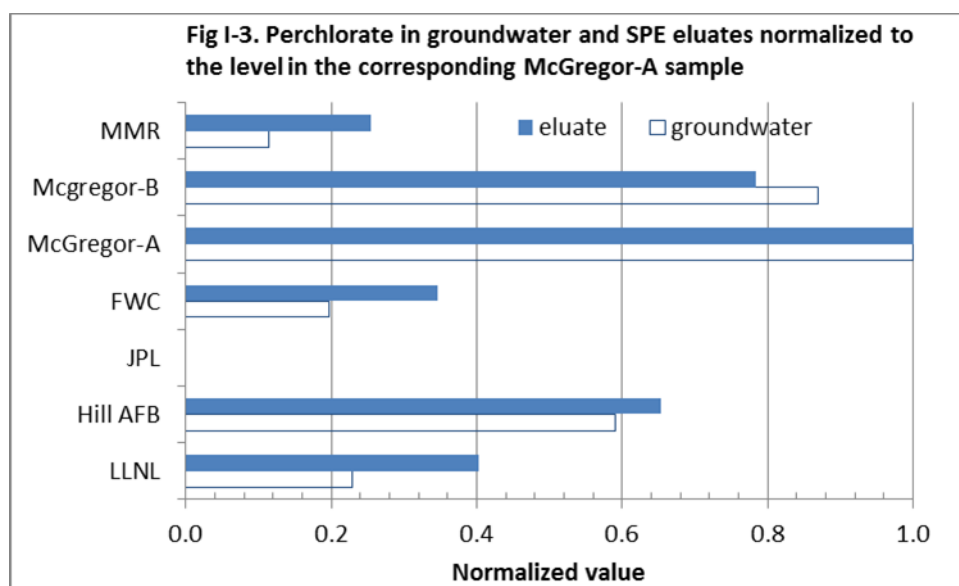
Figure I-3. Perchlorate in groundwater and SPE eluates detected by IC. These samples had previously been analyzed by ICPMS for uranium (see Table I-4 and Fig. I-1) and other elements (Tables I-1, I-2 and I-3).
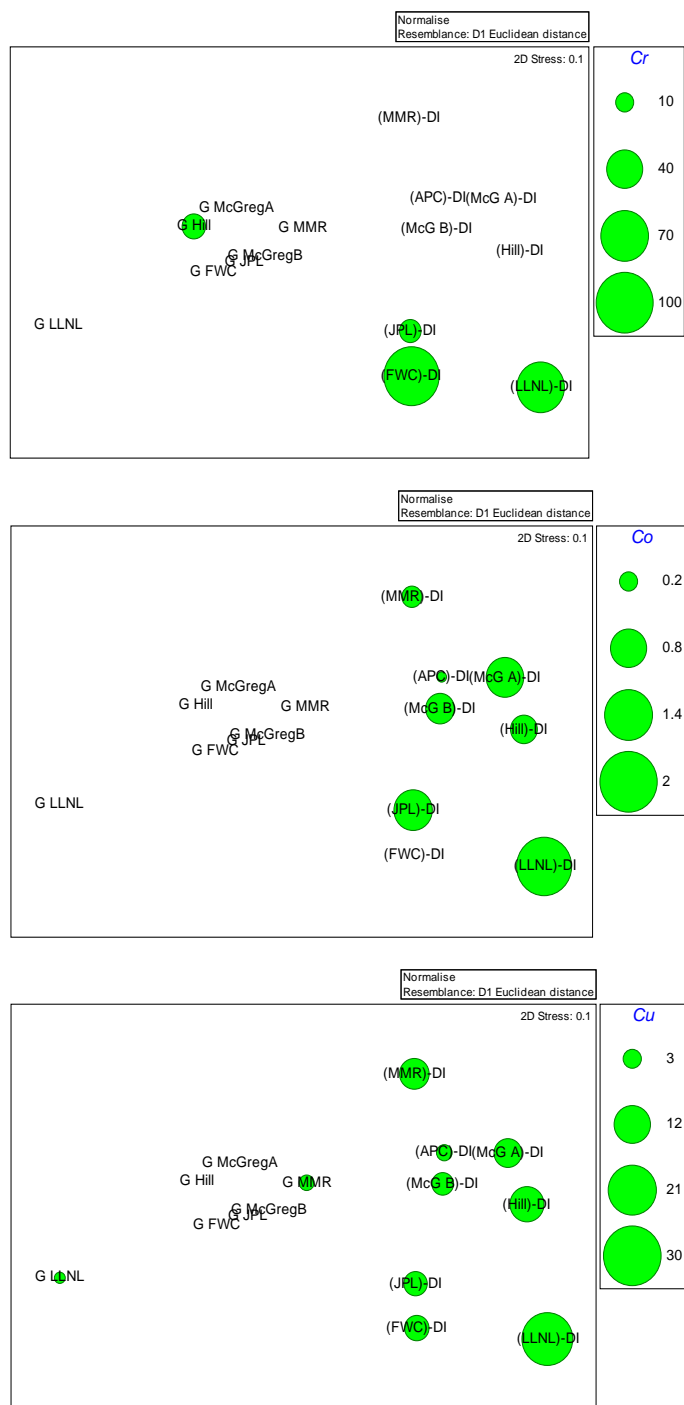
Figure I-4. Elements that are concentrated by the SPE step. An MDS plot was obtained based on normalized ICPMS data for elements in groundwater sources (denoted with a "G" prefix) and their corresponding SPE eluates. Bubble plots were obtained by superimposing circles of increasing size that represent increasing element concentrations. A different element is featured in each panel. The scale in the legends show the actual concentration of elements in groundwater (µg/L) or the difference between the element concentration in an eluent minus its concentration in the deionized water eluate blank. Elements shown in different panels are Cr, Co, Cu, Mo and U
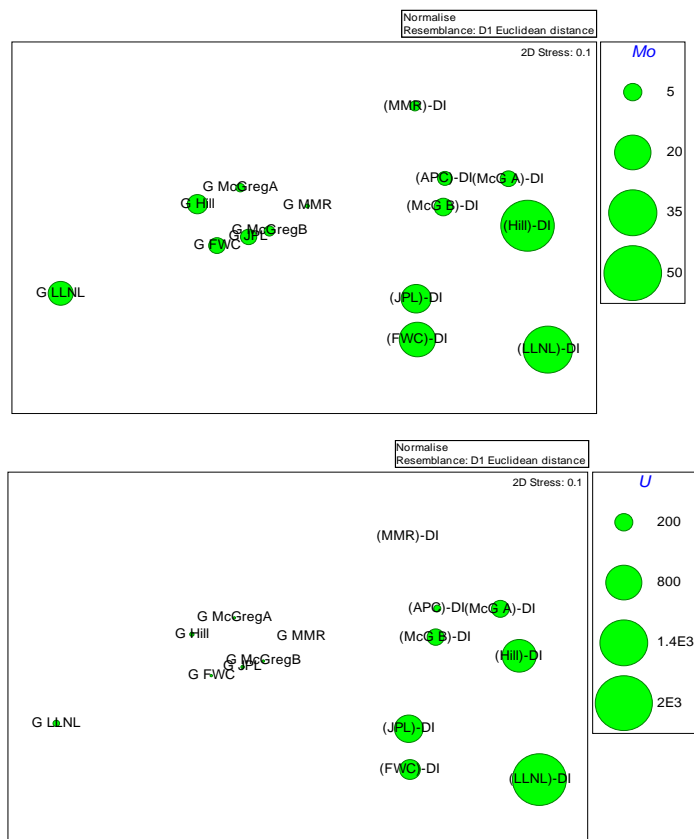
Figure I-4, continued. Elements that are concentrated by the SPE step
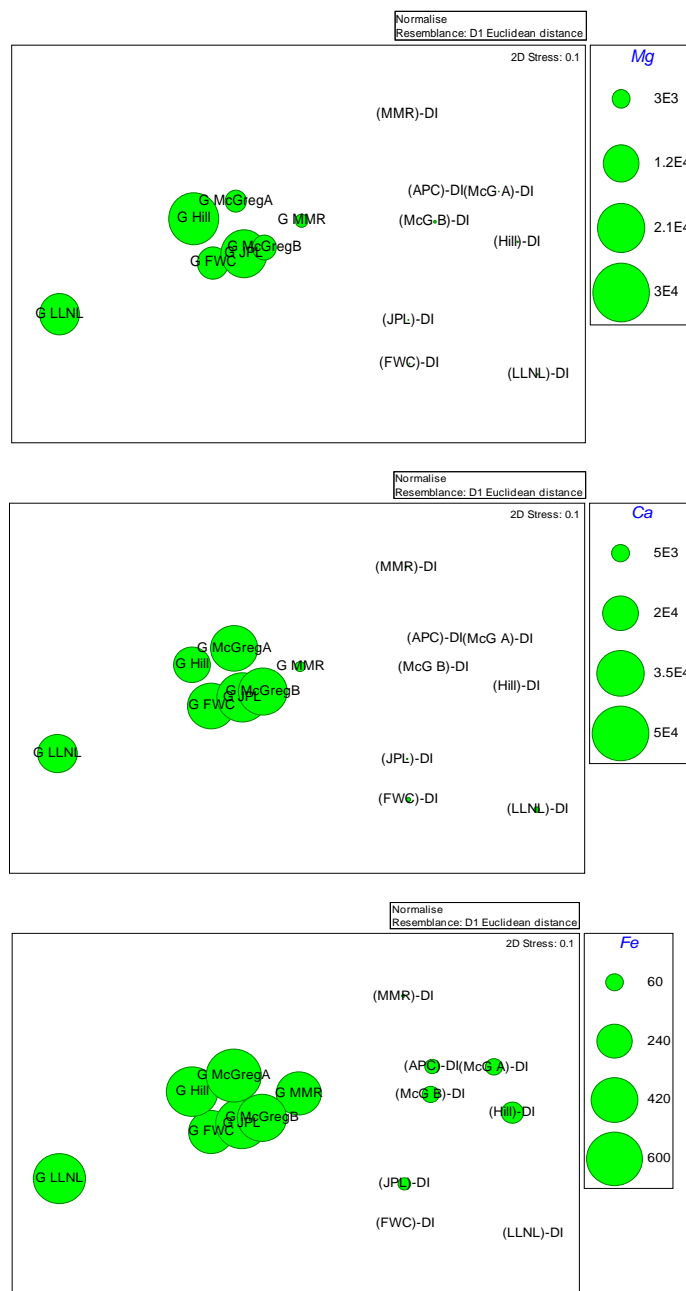
Figure I-5. Elements that are at lower levels in SPE eluates than in the corresponding groundwater source. Bubble plots were obtained by superimposing, on the MDS plot described in the Fig. I-4 legend, circles of increasing size that represent increasing element concentrations. A different element is featured in each panel. The scale in the legends show the actual concentration of elements in groundwater (µg/L) or the difference between the element concentration in an eluent minus its concentration in the deionized water eluate blank. Elements shown in different panels are Mg, Ca, and Fe

Appendix J: TESTING TO DETERMINE WHETHER URANIUM (VI) INTERFERES WITH THE BENCHTOP BIOASSAY

To test whether uranium (VI) would have an effect on perchlorate reductase activity in the benchtop bioassay, reaction mixtures containing 40 µM perchlorate and 0, 1, 2, 3, 5, 8, or 10 µM uranium (VI) were analyzed by the benchtop bioassay. These uranium concentrations cover the range observed in SPE eluates of groundwater (Tables I-4 and I-5). Results for the reaction mixtures are shown in Figure J-1 and listed in Table J-1 . There was no apparent difference in perchlorate reductase activity absence or presence of uranium (VI).

Table J-1. Benchtop bioassay perchlorate reductase activity in the presence of various amounts of uranium (VI)

Reaction category	Reaction mixture description	Concentration in reaction mixtures		Perchlorate reductase activity[a]		
		ClO_4^- (µM)	U(VI) (µM)	Rate (AU/min)	Rate – negative control rate (AU/min)	r^2 for rate regression lines
Controls	negative control	0	0	0.0015	0.0000	0.9896
	10 µM U, replicate 1	0	10	0.0015	0.0000	0.9829
	10 µM U, replicate 2	0	10	0.0013	-0.0002	0.9958
Test mixtures	40 µM per, 0 µM U	40	0	0.0110	0.0095	0.9850
	40 µM per, 1 µM U, replicate 1	40	1	0.0110	0.0095	0.9851
	40 µM per, 1 µM U, replicate 2	40	1	0.0113	0.0098	0.9873
	40 µM per, 2 µM U, replicate 1	40	2	0.0110	0.0095	0.9863
	40 µM per, 2 µM U, replicate 2	40	2	0.0108	0.0093	0.9867
	40 µM per, 3 µM U, replicate 1	40	3	0.0113	0.0098	0.9862
	40 µM per, 3 µM U, replicate 2	40	3	0.0115	0.0100	0.9805
	40 µM per, 5 µM U, replicate 1	40	5	0.0112	0.0097	0.9840
	40 µM per, 5 µM U, replicate 2	40	5	0.0116	0.0101	0.9853
	40 µM per, 8 µM U, replicate 1	40	8	0.0117	0.0102	0.9837
	40 µM per, 8 µM U, replicate 2	40	8	0.0113	0.0098	0.9863
	40 µM per, 10 µM U, replicate 1	40	10	0.0120	0.0105	0.9857
	40 µM per, 10 µM U, replicate 2	40	10	0.0144	0.0129	0.9665
	Test mixtures average ± SD				0.0100 ± 0.0009	

[a]Perchlorate reductase activity was measured as the 340 nm absorbance (AU) change per minute due to NADH oxidation. Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 5/8/2012 extract.

DESCRIPTION OF URANIUM SOLUTION AND MODIFIED ENZYME BUFFER USED FOR THE TESTING

The uranium (VI) solution added to reaction mixtures was prepared from a laboratory stock solution of uranium dichloride oxide trihydrate ($UO_2Cl_2 – 3 H_2O$) in deionized water. This was

further diluted in a pH 7.3 buffer containing 45 mM MOPS and 30 mM NaHCO$_3$ to obtain a 100 µM solution, which was added to the reaction mixtures.

The buffer used for the benchtop bioassay was the same as a LRB (i.e. laboratory reagent blank). It was prepared by applying 200 mL of deionized water to each of 25 SPE cartridges that had been conditioned with DTAB (decyltrimethyl ammonium bromide). After rinsing cartridges with a mixture of acetone and DTAB, and eluting with alkaline MOPS-NaCl by the standard procedure, eluates were pooled, neutralized to pH 7.1 by addition of 1 M HCl, and stored at 2-8 °C until use.
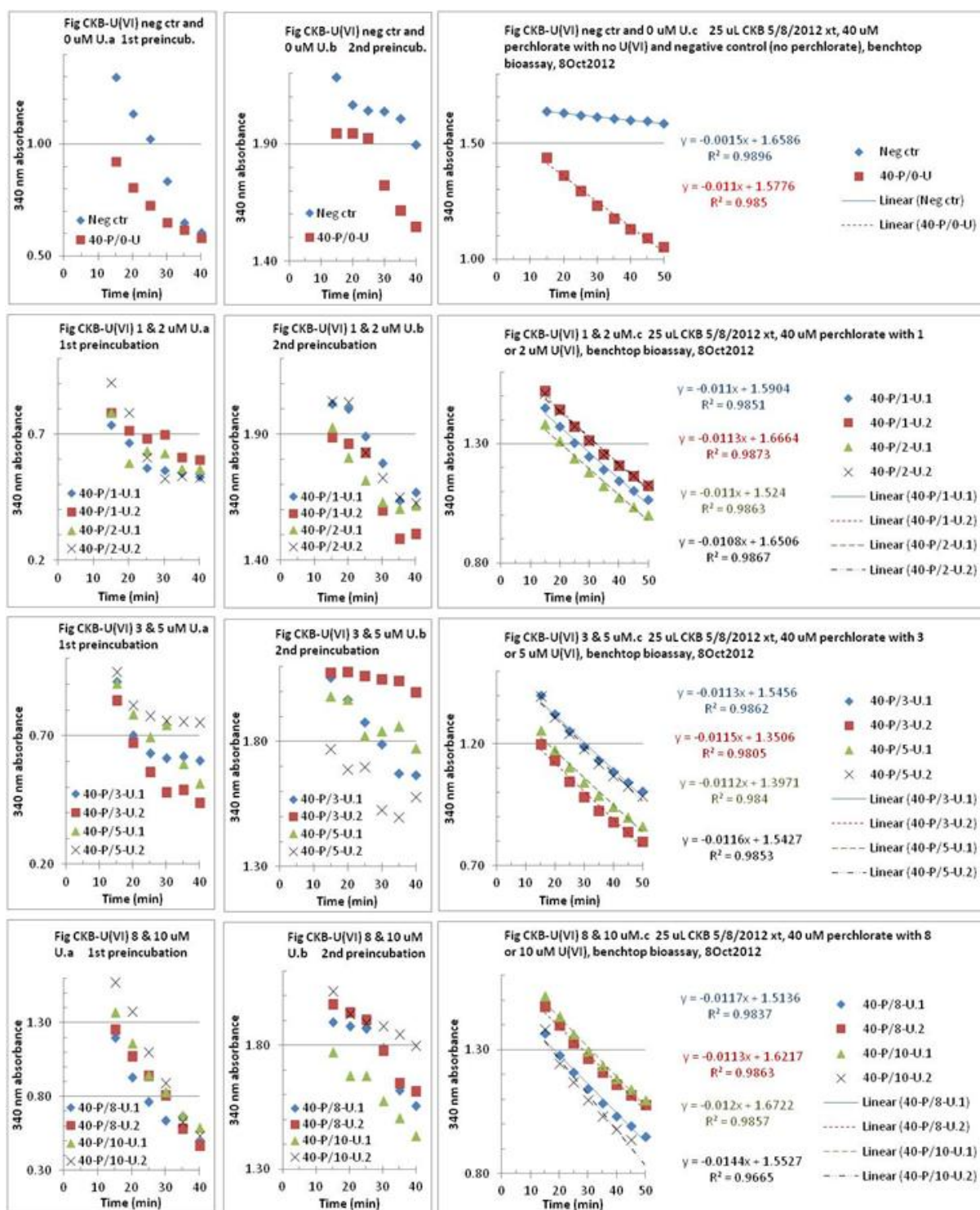
Figure J-1. Benchtop bioassay testing to determine the effect of uranium (VI) on perchlorate reductase activity. Reaction mixtures contained 40 μM perchlorate and 0, 1, 2, 3, 5, 8, or 10 μM of uranium (VI). There were two preincubation intervals before the reactions were initiated by the addition of cell extract. NADH was added after each preincubation period.

Appendix K. SOLID PHASE EXTRACTION OF PERCHLORATE USING LARGE RESIN BEADS OF STYRENE DIVINYLBENZENE

Preliminary testing was done to evaluate using gravity flow over large beads of styrene divinylbenzene (SDVB) to extract perchlorate from a solution. Syrene divinylbenzene (20 % cross-linked) white beads, 20 – 60 mesh (Strem Chemical, Inc.) were used. The efficiency of perchlorate extraction was compared to that seen with standard SPE cartridges.

A 3 g amount of large SDVB beads was placed in the barrel of a 10 mL syringe and held in place with glass wool placed at the bottom and top of the bead bed. The bead bed was rinsed sequentially with 150 mL acetone, then 300 mL deionized water, and then conditioned with 15 mL of 25 mM DTAB. After each liquid had passed through the column, air was forced through the bed several times to remove residual fluid trapped in the bed.

For comparison, solid phase extraction cartridges (Phenomenex, Inc., Torrance, CA, USA) containing 200 mg of SDVB resin were prepared for use by the standard procedure of rinsing each cartridge sequentially with 10 mL acetone, then 20 mL deionized water, and conditioning with 1 mL of 25 mM DTAB. After each liquid had passed through, air was forced through the cartridge to remove residual fluid trapped in the resin bed.

200 mL of perchlorate solution (100 µg/L) was applied with gravity flow to triplicate large bead columns and the effluent flow-through solutions were collected for subsequent perchlorate analysis by IC. In parallel, 200 mL of the perchlorate solution was applied to triplicate SPE cartridges by the standard method of applying pressure from a nitrogen compressed gas cylinder to force the solution through the resin at a moderate flow rate. The effluent solutions were collected for subsequent perchlorate analysis.

Table K-1 shows the efficiency of perchlorate removal from the effluent by the gravity flow procedure and removal by the standard SPE cartridge method. The percent of perchlorate extracted by the gravity flow procedure was 96.1 ± 1.6 (average of 3 syringes ± standard deviation), compared to 100 percent with the standard SPE cartridge procedure whereby samples are applied under pressure.

Table K-1. Perchlorate extraction by gravity flow through columns of large SDVB beads compared to SPE SDVB cartridges with sample applied under pressure

Sample description	Perchlorate (µg/L)	Estimated percent of perchlorate extracted[a]
Perchlorate solution applied to large bead columns and SPE cartridges		
Replicate 1	87.7	NA
Replicate 2	88.1	NA
Replicate 3	88.5	NA
Average ± SD	88.1 ± 0.4	
Effluent from large bead SDVB columns		
Column 1	1.9	97.9
Column 2	4.6	94.7
Column 3	3.8	95.7
Average ± SD	3.4 ± 1.4	96.1 ± 1.6
Effluent from SPE cartridges		
Cartridge 1	None detected	100
Cartridge 2	None detected	100
Cartridge 3	None detected	100

[a]NA denotes not applicable

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Appendix L: ASPECTS OF BIOASSAY TESTING

L-1.0 PREPARATION OF CELL EXTRACTS

Extracts of strain CKB are identified by the date of preparation. Those used in benchtop and plate reader bioassay testing and are listed in tables L-2 and L-3, respectively.

The 12/10/2007 extract was prepared as previously described[19]. A similar procedure was used to prepare the 8/3/2011 and 5/8/2012 extracts.

Briefly, cell cultures were grown anaerobically on phosphate medium containing 10 mM acetate and 10 mM chlorate. Cells in late exponential growth phase were harvested by centrifugation. The cell pellet was resuspended in anaerobic MOPS buffer (50 mM, pH 7.2) by vortexing inside an anaerobic chamber. The cell suspension was repelleted and the cell pellet was stored in a – 80 °C freezer.

To prepare extracts, a thawed cell pellet was suspended in 50 mM MOPS (pH 7.2) anaerobic buffer inside an anaerobic chamber. Prior to use, the buffer was amended with sufficient DNase (Sigma-Aldrich Corporation, DNase I from bovine pancreas) and lysozyme (Thermo Scientific Pierce, product no. 89833) to obtain final concentrations of 0.01 mg DNase/mL and 0.1 mg lysozyme/mL in the cell suspension. The amount of this buffer used was 5 mL per gram of cell pellet wet weight. The mixture of cells and degradative enzymes was incubated at room temperature for about 30 to 60 minutes before the sonication step. The tube with the cell suspension was transferred from the anaerobic chamber to a laboratory bench, placed in ice bucket, and flushed with N_2 prior to disruption by sonication. Cells were disrupted by 5 to 6 sonication bursts (30 seconds each) with a 30 second pause between bursts.

An anaerobic chamber was used to transfer the sonicated material to polycarbonate centrifuge tubes (capped with a three-part screw cap assembly). Tubes were centrifuged in a preparative ultracentrifuge using a Ti 70.1 rotor for 1 hour at 35000 rpm (i.e. 112,000 x g). After centrifugation, tubes were opened inside an anaerobic chamber. The clear upper portion of the supernatant was removed with a Pasteur pipette, mixed with anaerobic glycerol to a final concentration of about 8 %, and aliquots were distributed to microfuge tubes. The tubes with freshly prepared extract were removed from the anaerobic chamber and flash frozen in liquid N_2. The tubes with frozen extract were stored in a – 80 °C freezer until use.

L-2.0 BENCHTOP BIOASSAY PROCEDURE

L-2.1   SET-UP CUVETTES FOR BENCHTOP BIOASSAY

Prepare a dilution of CKB extract to be added to reaction mixtures. In an anaerobic chamber, dilute the extract 1/3 or 1/4-fold in anaerobic 50 mM MOPS (pH 7.2) buffer contained in a Hungate tube. Add NADH and PMS to the buffer prior to adding extract to help further reduce the buffer. The amount of NADH and PMS added should bring their final concentrations in the

diluted extract to the same concentrations as are used in cuvette reaction mixtures (see below). Store diluted extract in an ice bucket until use.

Deliver 1 mL of SPE eluate (neutralized to about pH 7.2 with 1N HCl) to a cuvette, add 16 µL NADH (45 mM in 0.01 M NaOH), and overlay with 0.9 mL of mineral oil. For a perchlorate standards reaction, deliver 0.95 mL of enzyme buffer (200 mM MOPS, 2M NaCl, adjusted to pH 7.2 with HCl) to a cuvette, add 50 µL of deionized water (for the negative control) or 20-fold concentrated solution of perchlorate standard, 16 µL NADH, and overlay with 0.9 mL of mineral oil.

Deliver 32 µL of PMS (4 – 5 mM in deionized water) to the aqueous phase below the mineral oil overlay. Mix. Follow absorbance change at 340 nm at 5 minute intervals to monitor the non-enzymatic oxidation of NADH. After 45 – 50 minutes, calculate how much NADH should be added to the cuvette to restore the 340 nm absorbance to about 2.0. Add the NADH below the mineral oil overly, mix, and again monitor the non-enzymatic oxidation of NADH for 45 – 50 minutes. Again calculate how much NADH to add to restore the 340 nm absorbance to about 2.0.

Add the NADH. Then add 0.1 mL of diluted CKB cell extract below the mineral oil overlay using a 1 mL syringe to transfer diluted extract from the Hungate tube to the reaction mixture. Mix. Measure the 340 absorbance every 5 minutes for about 50 minutes to monitor the progress of the perchlorate reductase reaction.

The millimolar extinction coefficient for NADH at a wavelength of 340 nm is 6.22. The extinction coefficient was used to convert 340 nm absorbance units to µmoles of NADH oxidized per mL of reaction mixture. Semi-micro quartz cuvettes with a 1 cm light path that accommodate a nominal volume of 1.4 mL, but which have a standard threaded top so that the actual total capacity is about 2.5 mL, were used (Starna Cells, Inc).

L-2.2   RECORD OF NADH SUPPLEMENTATION MADE TO BENCHTOP BIOASSAY REACTIONS

As discussed in the text of this report, preincubation of reaction mixtures containing NADH and PMS was used to reduce SPE eluate (as well as negative and perchlorate standard control) reactions prior to the addition of extract. Reaction mixtures need to be mixed by stirring or repipetting after additions are made in order to obtain homogeneity. The mixing process unavoidably also introduces some oxygen into the mixture.

Table L-1 lists the average amount of NADH supplement added to reactions for each benchtop bioassay batch. Also listed is the number of times the reaction mixtures were mixed in order to achieve homogeneity. For instance, reactions were mixed for the first time immediately after adding PMS, and 340 nm absorbance was then monitored for the first preincubation interval.. For batches tested with all sources except Hill AFB and McGregor NWIRP, NADH supplementation was performed after the first preincubation, after which reactions were mixed for the second time, and absorbance monitored again for the second preincubation interval. An additional NADH supplement was added after the second preincubation, followed immediately by addition of diluted extract, and reaction mixtures were then mixed for the third time.

Absorbance readings after extract addition provide the data for determining the rate of enzyme activity (AU change/minute). For Hill AFB and McGregor NWIRP testing, the number of NADH supplements, the number of times reaction mixtures were mixed, and the number of preincubation intervals varied as indicated in Table L-1.

Table L-1. Supplementation of benchtop bioassay reaction mixtures with NADH at the end of preincubation intervals and the total number of times reaction mixtures were mixed

Batch	SPE eluates				Negative and standard controls			
	No. of supple-ments	No. of times mixed	Average[a] ± SD (µL)	No. of reac-tions	No. of supple-ments	No. of times mixed	Average[a] ± SD (µL)	No. of reac-tions
102, Hill AFB[b]								
1 (11Aug2011)	2	3	10.0 ± 0.76	15	0	1	4.0 ± 0.0	2
2 (11Aug2011	1	3[c]	7.7 ± 0.70	15	0	1	0	2
3 (12Aug2011)	2	3	13.1 ± 1.03	15	0	1	0	2
103, McGregor NWIRP[d]								
1 (6Nov2011)	1	2	8.9 ± 0.40	15	1	2	4.9 ± 0.17	3
2 (9Nov2011)	1	2	8.7 ± 0.72	15	1	2	4.1 ± 0.75	3
104, JPL								
1 (9Dec2011)	2	3	10.3 ± 0.60	15	2	3	4.3 ± 0.30	3
2 (12Dec2011)	2	3	9.3 ± 0.87	15	2	3	3.8 ± 0.49	3
3 (12Dec2011)	2	3	7.8 ± 0.91	15	2	3	6.5 ± 0	3
4 (16Dec2011)	2	3	11.4 ± 0.61	9	2	3	5.5 ± 0.45	3
105, FWC								
1 (16Feb2012)	2	3	12.0 ± 0.60	15	2	3	4.6 ± 0.52	3
2 (18Feb2012)	2	3	11.8 ± 0.98	15	2	3	6.1 ± 0.15	3
3 (20Feb2012)	2	3	12.2 ± 0.42	15	2	3	6.5 ± 0.35	3
106, MMR								
1 (15Mar2012)	2	3	9.6 ± 1.55	15	2	3	6.2 ± 0.20	3
2(18Mar2012)	2	3	7.7 ± 2.20	15	2	3	6.0 ± 0	3
3 (19Mar2012)	2	3	10.9 ± 1.24	15	2	3	6.3 ± 0.92	3
107, LLNL								
1 (20Apr2012)	2	3	12.7 ± 1.13	15	2	3	5.7 ± 0.10	3
2 (23Apr2012)	2	3	10.8 ± 2.06	15	2	3	5.6 ± 0.23	3
3 (24Apr2012)	2	3	9.2 ± 1.63	15	2	3	6.0 ± 0.35	3

[a]Values are the average µL of NADH stock solution added to reaction mixtures at the end of both preincubation intervals

[b]Only reaction mixtures containing SPE eluates were supplemented with additional NADH. That is, NADH was not added to negative and positive control reaction mixtures after the preincubation intervals

[c]For the Hill AFB SPE eluates B1-(0 spike) and B3-(0 spike), reactions were only mixed 2 times instead of 3. The other SPE elutate reactions were mixed 3 times

[d]There was only one preincubation interval used for McGregor testing. NADH was added at the end of the preincubation period prior to the addition of cell extract

L-2.3   PARAMETERS FROM REGRESSION LINES OBTAINED WITH PERCHLORATE STANDARDS (0, 20, 40 µM) IN BENCHTOP BIOASSAY TESTING

Negative and positive control reactions were used to check that perchlorate reductase activity was present for each batch of reactions. The rate (change in absorbance units/minute) for the negative control was subtracted from the rate of reaction for SPE eluates or perchlorate standards. Regression lines for the rate of absorbance change for standards (minus the rate for the negative control) were calculated and the slope and r^2 values are listed in Table L-2. Figures showing the regression lines for perchlorate controls are in Figures B-102, 103, 104, 105, 106, and 107. Standards regression lines were used to calculate the x-intercepts listed in the table. Clearly, r^2 correlation coefficients were high when standards were tested in the benchtop bioassay, and the average error for x-intercepts was low, i.e. $\pm$ 1.6 µM perchlorate (n = 15).

Table L-2. Results from benchtop bioassay testing of perchlorate standards[a,b]

Row	Batch ID	Test date	x-intercept		Slope parameters		r^2	Extract/reaction (µL), and CKB extract date
			(µM)	± error (µM)	slope	± error		
1	Hill #1	11Aug2011	ND	NA	0.000108	NA	1.0	33, 8/3/2011
2	Hill #2	11Aug2011	ND	NA	0.000135	NA	1.0	33, 8/3/2011
3	Hill #3	12Aug2011	ND	NA	0.000130	NA	1.0	33, 8/3/2011
4	McG #1	6Nov2011	1.4	3.1	0.000120	0.000014	0.9857	33, 8/3/2011
5	McG #2	9Nov2011	-1.3	2.8	0.000105	0.000012	0.9881	33, 8/3/2011
6	JPL #1	9Dec2011	0.0	0.0	0.000115	0.000000	1.0000	33, 8/3/2011
7	JPL #2	12Dec2011	0.95	2.1	0.000123	0.000010	0.9932	33, 8/3/2011
8	JPL #3	12Dec2011	0.56	1.2	0.000120	0.000006	0.9977	33, 8/3/2011
9	JPL #4	16Dec2011	0.71	1.6	0.000118	0.000007	0.9962	33, 8/3/2011
10	FWC #1	16Feb2012	-0.055	0.12	0.000151	0.0000007	1.0000	25, 12/10/2007
11	FWC #2	18Feb2012	-0.74	1.7	0.000158	0.000010	0.9959	25, 12/10/2007
12	FWC #3	20Feb2012	-1.2	2.8	0.000166	0.000018	0.9882	25, 12/10/2007
13	MMR #1	15Mar2012	0.90	2.0	0.000132	0.000010	0.9940	25, 12/10/2007
14	MMR #2	18Mar2012	-0.38	0.85	0.000175	0.000006	0.9989	25, 12/10/2007
15	MMR #3	19Mar2012	0.097	0.22	0.000173	0.000001	0.9999	25, 12/10/2007
16	LLNL #1	20Apr2012	-0.76	1.7	0.000175	0.000012	0.9957	25, 12/10/2007
17	LLNL #2	23Apr2012	-1.2	2.7	0.000169	0.000018	0.9889	25, 12/10/2007
18	LLNL #3	24Apr2012	-0.56	1.3	0.000189	0.000009	0.9976	25, 12/10/2007
		Average	-0.11	1.61				

[a]Slope is the 340 nm absorbance/minute/µM perchlorate. The negative control rate (AU/min) was subtracted from rate values before the regression line was plotted. Negative control rates for each batch are listed in the footnotes to Appendix B Tables B-102, 103, 104, 105, 106, and 107. Parameters of slope and r^2 are from Excel regression analysis (LINEST function).

[b]For the Hill batches, only negative control and 40 µM perchlorate standard reactions were tested. Hence, no regression line could be determined and the "slope" is only estimated from the two data points: 0 and 40 µM. For all other testing, three data points were used to generate regression lines: 0 (i.e. negative control), 20 and 40 µM perchlorate standards. ND denotes not determined, NA denotes not applicable

L-3.0 ANAEROBIC PLATE READER BIOASSAY PROCEDURE

L-3.1 SET-UP MICROPLATE WELLS FOR PLATE READER BIOASSAY

Deliver 276 µL of SPE eluate (neutralized to about pH 7.2 with 1 N HCl) to a microplate well. Add 4 µL NADH (45 mM in 0.01 M NaOH), and 10 µL PMS (4 – 5 mM in deionized water and mix with the tip of the pipettor. Preincubate for 45 to 60 minutes to be sure the SPE eluate is non-enzymatically reduced by the NADH/PMS system. Add 10 µL CKB cell extract and mix again. By contrast with the benchtop bioassay format, the CKB extract is not diluted before adding to reaction mixtures. Furthermore, reaction mixtures are not supplemented with additional NADH after the preincubation interval. The 340 nm absorbance change is measured for 20 to 45 minutes to determine the rate of the perchlorate reductase reaction, using the kinetics program of the plate reader.

A negative control (i.e. no perchlorate present) should be included in each batch and the rate of NADH oxidation for the negative control subtracted from the rate of reaction for all other reaction mixtures. Positive control reaction mixtures containing perchlorate standards should also be included with each microplate batch of reactions. For the negative and positive control reactions, deliver 261 µL of enzyme buffer (200 mM MOPS, 2 M NaCl, adjusted to pH 7.2 with HCl) to a well. Add 15 µL of deionized water (negative control) or 15 µL of a 20-fold concentrated solution of perchlorate standard, 4 µL NADH, and 10 µL PMS. Mix the contents of the well, and preincubate. Monitor the 340 nm absorbance change during preincubation. Add 10 µL CKB cell extract and mix again. Measure the 340 nm absorbance change for 20 to 45 minutes to determine the rate of the perchlorate reductase reaction.

Costar 96-well non-sterile acrylic microplates with a UV transparent flat bottom were used (Corning, Inc.). The final volume of reaction mixtures was 0.3 mL per well. The relationship between 340 nm absorbance and NADH concentration when the well volume is 0.3 mL was empirically determined to be 4.959 absorbance units per mM. This factor was used to convert 340 nm absorbance units to µmoles of NADH oxidized per mL of reaction mixture.

L-3.2 PARAMETERS FROM REGRESSION LINES OBTAINED WITH PERCHLORATE STANDARDS (0, 10, 20, 30, 40 µM) IN PLATE READER BIOASSAY TESTING

The rate (change in absorbance units/minute) for the negative control was subtracted from the rate of reaction for wells containing SPE eluates or perchlorate standards. Regression lines for the rate of absorbance change for standards (minus the rate for the negative control) were calculated and parameters for these are listed in Table L-3. Figures showing the regression lines for the perchlorate controls are in Figures F-1, 4, and 5. Standards regression lines were used to calculate the x-intercepts listed in the table. The r^2 correlation coefficients were high when standards were tested in the plate reader bioassay, and the average error for x-intercepts was low, i.e. $\pm$ 0.64 µM perchlorate (n = 3).

Table L-3. Results from plate reader bioassay testing of perchlorate standards[a,b]

Row	Batch ID	Test date	x-intercept		Slope parameters		r^2	Extract/reaction (µL), and CKB extract date
			(µM)	± error (µM)	Slope	± error		
1	Batch 1	5Aug2011	-0.56	0.65	0.000056	0.000001	0.9979	10, 8/3/2011
2	Batch 2	29Jun2012	ND	NA	ND	NA	NA	10, 5/8/2012
3	Batch 3	6Jul2012	ND	NA	ND	NA	NA	10, 5/8/2012
4	Batch 4	11Jul2012	0.81	0.95	0.000326	0.000013	0.9955	10, 5/8/2012
5	Batch 5	11Jul2012	0.44	0.33	0.000306	0.000004	0.9995	10, 5/8/2012
		Average	0.23	0.64				

[a]Slope is the 340 nm absorbance/minute/µM perchlorate. The negative control rate (AU/min) was subtracted from rate values before the regression line was plotted. Negative control rates for each batch are listed in the footnotes to Appendix F Tables F-2, 3, 4, 5, and 6. Parameters of slope and r^2 are from Excel regression analysis (LINEST function).

[b]For batch1 duplicate wells were tested with 0, 10, 20, 30, and 40 µM of perchlorate, and the regression line was plotted with the average of the duplicate wells. No regression line was plotted for batches 2 and 3 – because, by mistake, the perchlorate standards added were below the limit of detection; these wells were averaged and used as the negative control rate. For batches 4 and 5, single wells with 0, 10, 20, 30, or 40 µM perchlorate were plotted to obtain the standard curves.

L-4.0   CONFIRMATION OF STOICHIOMETRY OF NADH OXIDIZED TO PERCHLORATE REDUCED IN BENCHTOP AND PLATE READER BIOASSAYS

The stoichiometry of the NADH-coupled perchlorate reductase assay was previously studied[19] and has been described by Heinnickel et al.[2]. Confirmatory testing was performed with the CKB 5/8/2012 extract using both the benchtop and plate reader formats of the bioassay. The initial concentration of perchlorate in reaction mixtures was 40 µM.

For the benchtop bioassay, the equivalent of 25 µL of undiluted extract was used for each reaction mixture. Figure L-1.i. shows the 340 nm absorbance for the negative control (no perchlorate added to the cuvette) and the 40 µM perchlorate reaction mixture for a 45 minute
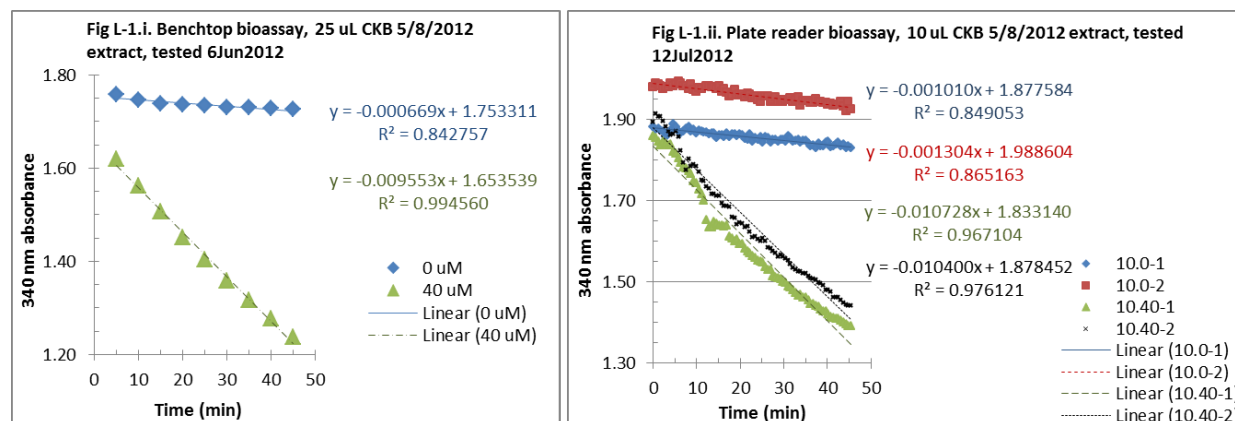

Figure L-1. 340 nm absobance change for benchtop or plate reader bioassays using the CKB 5/8/2012 extract. (i) Data from the benchtop bioassay is in the left hand panel, **(ii)** the right hand panel has the data from the plate reader bioassay

incubation period. To measure perchlorate reduction directly, a second reaction mixture with 40 µM perchlorate was tested in parallel. At 10 minute intervals, 60 µL samples were removed and diluted 100-fold to stop the enzyme reaction. Perchlorate concentration was subsequently determined by IC in the diluted samples. Table L-4 lists the perchlorate reduced and NADH oxidized in the parallel benchtop bioassay reaction mixtures over a 40 minute incubation period. Figure L-2.i shows the plot of these data. The slope corresponds to the stoichiometry for the µmoles of NADH oxidized per µmole of perchlorate reduced. The parameters for the regression line were calculated, and are also listed in Table L-4.

Table L-4. Perchlorate reduced and NADH oxidized in parallel reaction mixtures for benchtop and plate reader bioassays

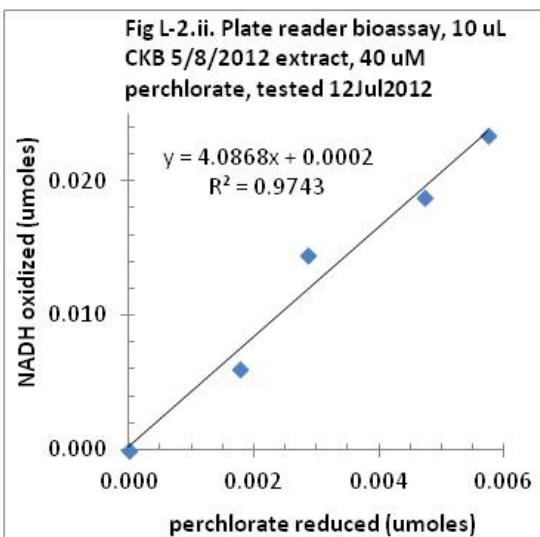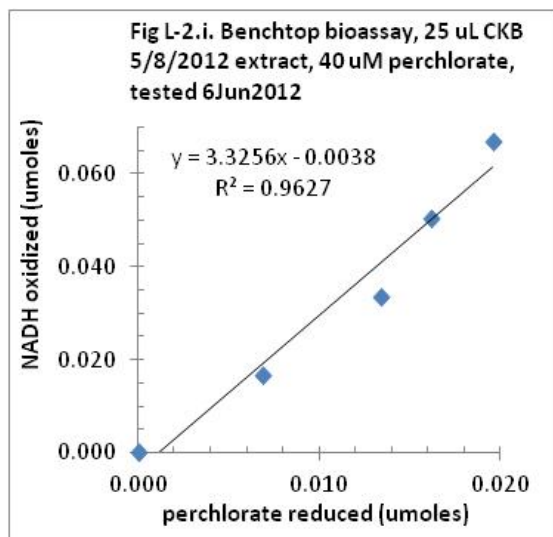| Benchtop bioassay tested 6Jun2012			Plate reader bioassay tested 12Jul2012		
Time (min)	Perchlorate reduced (µmoles)	NADH oxidized (µmoles)	Time (min)	Average Perchlorate reduced (µmoles)	Average NADH oxidized (µmoles)
0	0.0000	0.0000	0	0.00000	0.00000
10	0.0069	0.0167	10	0.00176	0.00604
20	0.0134	0.0335	20	0.00285	0.01449
30	0.0162	0.0502	30	0.00471	0.01881
40	0.0196	0.0670	40	0.00575	0.02340
Regression parameters for benchtop or plate reader bioassay results					
Slope ± error	3.3256 ± 0.3781		4.0868 ± 0.3834		
y-intercept ± error	-0.0038 ± 0.0050		0.0002 ± 0.0014		
r^2	0.9627		0.9743		

Figure L-2. Stoichiometry of perchlorate reduction and NADH oxidation in benchtop and plate reader bioassays. (i) Data from the benchtop bioassay is in the left hand panel, **(ii)** the right hand panel has the data from the plate reader bioassay

For the plate reader bioassay, 10 µL of extract was used for each reaction well. Figure L-1.ii shows the 340 nm absorbance for duplicate negative control and 40 µM perchlorate reaction mixtures over an incubation period of about 45 minutes. In parallel, a second microplate was used to measure perchlorate reduction directly. This plate had 12 replicate wells containing reaction mixtures with 40 µM perchlorate. Each well was sampled only once. At 10 minute intervals, duplicate 60 µL samples were diluted 100-fold to stop the enzyme reaction. Perchlorate concentration was subsequently determined by IC in the diluted samples. Table L-4 lists the perchlorate reduced and NADH oxidized in the parallel bioassay reaction mixtures. Figure L-2.ii shows the plot of these data, with the slope corresponding to the stoichiometry for the µmoles of NADH oxidized per µmole of perchlorate reduced. The parameters for the regression line were calculated, and are also listed in Table L-4.

The observed stoichiometry for µmoles of NADH oxidized per µmole of perchlorate reduced was 3.3 ± 0.38 with the benchtop bioassay and 4.1 ± 0.38 with the plate reader bioassay. As discussed in Section 2.1 above, a stoichiometry of 2 would be expected if only perchlorate reductase were active in reaction mixtures, and a stoichiometry of 4 is possible if chlorite dismutase is also active.

L-5.0   CONTRAST BETWEEN CKB AND RCB EXTRACTS REGARDING PERCHLORATE REDUCTASE RATES AT DIFFERENT PERCHORATE CONCENTRATIONS

The activity (340 nm absorbance change/minute) of extracts of *Dechloromonas agitata* strain CKB shows a clear dependance on the initial concentration of perchlorate (Coates and Achenbach, unpublished). Bioassay testing was also done with extracts of another strain in the *Dechloromonas* genus, *Dechloromonas aromatica* strain RCB, to determine whether a similar degree of dependence on initial perchlorate concentration would be observed.

Both strains were cultivated on the same medium, and the procedure described in Section L-1.0 was used to prepare extracts with both strains on 8/3/2011. The plate reader bioassay format was used as described in Section L-3.0, and the reactions with both strains were performed simultaneously in the same microplate. Perchlorate concentrations of 0, 10, 20, 40, and 500 µM were tested in triplicate for each extract. The reactions without perchlorate are the negative controls. Figure L-3 shows the 340 nm absorbance change for all the reactions over the course of about 46 minutes. The rates of absorbance change with the RCB extract were clearly biphasic for the lower perchlorate concentrations. The faster rate phase was from 0 to about 4 to 5 minutes of reaction for 10 µM perchlorate, from 0 to 11 minutes for 20 µM perchlorate, and from 0 to 31 minutes for 40 µM perchlorate. Rates for 500 µM perchlorate were not biphasic during the interval over which absorbance changes were monitored.
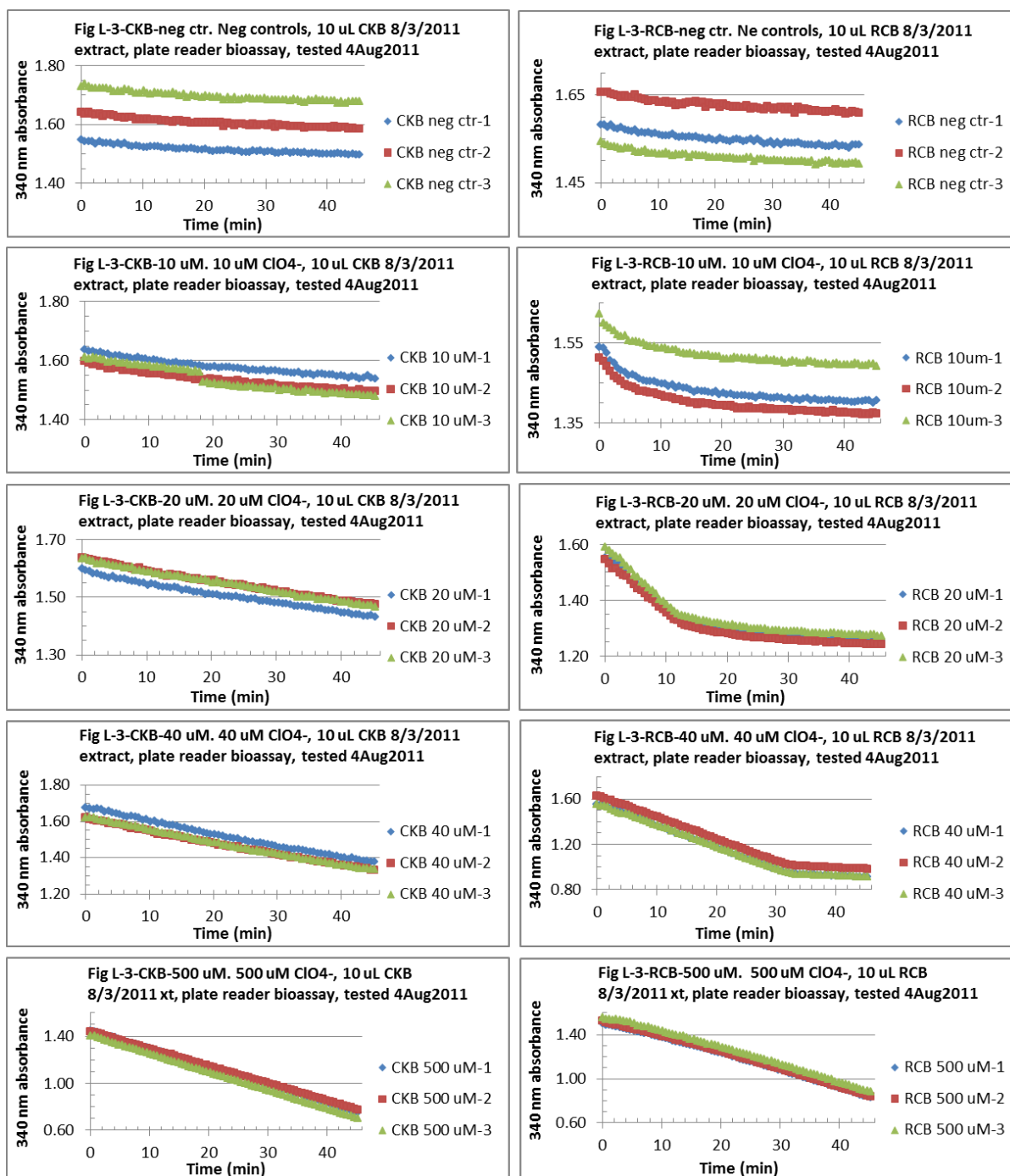
Figure L-3. 340 nm absorbance change with CKB and RCB extracts at different initial perchlorate concentrations

Table L-5 lists the average rates of 340 nm absorbance change for each test condition as well as the standard deviation for the replicates. Also listed are the average rates after subtracting the average negative control rate. With the RCB reactions, different time intervals were used to determine the absorbance change during the faster rate phase, and these intervals are also listed in the table. Since there was a small difference in the negative control rates depending on the time interval, negative control rates were calculated for the different intervals. These negative control rates these are also listed in the table.

Table L-5. Rates of perchlorate reductase activity with CKB and RCB extracts for various initial perchlorate concentrations

| Initial ClO4⁻ (µM)	CKB 8/3/2011 extract				RCB 8/3/2011 extract			
	Time interval (min)	Average rate		Average rate – neg. ctr	Time interval (min)	Average rate		Average rate – neg. ctr
		(AU/min)	SD	(AU/min)		(AU/min)	SD	(AU/min)
10	0 to 16.05	0.00290	0.00018	0.00106	0 to 4.2	0.01541	0.00253	0.01251
20	0 to 16.05	0.00423	0.00006	0.00239	0 to 10.1	0.01959	0.00119	0.01735
40	0 to 16.05	0.00722	0.00028	0.00538	0 to 29.7	0.01935	0.00016	0.01817
500	0 to 16.05	0.01516	0.00050	0.01332	0 to 45.3	0.01516	0.00012	0.01424
Negative controls								
0	0 to 16.05	0.00184	0.00009	0.00000	0 to 4.2	0.00290	0.00046	0.00000
0					0 to 10.1	0.00225	0.00014	0.00000
0					0 to 29.7	0.00118	0.00005	0.00000
0					0 to 45.3	0.00092	0.00005	0.00000
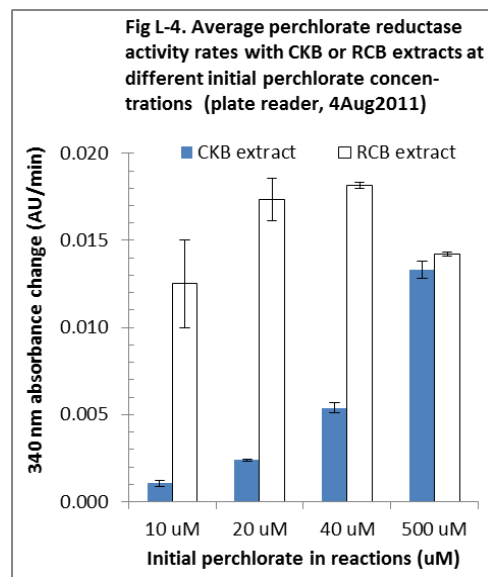
Figure L-4 shows a comparison of the average rates of absorbance change, after subtracting the average negative control rate, for each concentration of perchlorate tested with the two extracts. Had the development of a kit for the benchtop bioassay gone forward, it might have been advantageous to test RCB extracts for their suitability for such an application, since the rate of absorbance change is faster at low perchlorate concentrations when an RCB extract is used. The basis of the difference between the extracts might be due to an operationally lower K_m for perchlorate with the RCB strain.

Figure L-4. Perchlorate reductase rates with CKB or RCB extracts. Error bars show the standard deviation of triplicate determinations